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CONTENTS

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The Effect of Saline Washings of Isolated Jejunal Loops on Gastric Secretion. *William DeW. Andrus, Jere W. Lord, Jr., Paul Stefko and James A. Dingwall, III* 287

Studies on the Effect of Anoxic Anoxia on the Central Nervous System. *M. Kessler, H. Hailman and E. Gellhorn* 291

The Effect of Anoxic and Anemic Anoxia on the Leucocyte Count. *C. H. Cress, F. B. Clare and E. Gellhorn* 299

The Effects of Polycythemia and of a Carrot Diet on Resistance to Anoxia. *Paul Wetzig and Fred E. D'Amour* 304

Water and Fat Content of Orbital Tissues of Guinea Pigs with Experimental Exophthalmos Produced by Extracts of the Anterior Pituitary Gland. *George K. Smelser* 308

The Absence of Phosphate Transfer in Oxidative Muscular Contraction. *Jacob Sacks* 316

The Persistence of Heat Acclimatization in Man. *Austin Henschel, Henry Longstreet Taylor and Ancel Keys* 321

Some Effects of Pectin Solutions During Posthemorrhagic Hypotension. *Samuel Middleton and Carl J. Wiggers* 326

The Renal Excretion of Chloride by the Normal and by the Diabetes Insipidus Dog. *Ruth S. Hare, Kendrick Hare and Donald M. Phillips* 334

Increased Red Blood Cell Fragility after Fat Ingestion. *Joan Longini and Victor Johnson* 349

The Effect of Oxygen Deprivation on the Relation between Stimulus Intensity and the Latency of Visual After-Images. *R. A. McFarland, L. M. Hurvich and M. H. Halperin* 354

The Effect of Cyanide and other Metal Binding Substances on the Pharmacological Action of Epinephrine. *Jonas S. Friedenwald and Wilhelm Buschke* 367

Some Effects of Low Barometric Pressures on Kidney Function in the White Rat. *Herbert Silvette* 374

The Effects of Low Barometric Pressures on the Structure of the Kidneys of the White Rat. *James E. Kindred* 387

The Validity of "Ovulation Potentials". *James M. Snodgrass, John Rock and Miriam F. Menkin* 394

Rôle of the Kidneys in the Resistance of Rats to Hemorrhage. *Henry T. Bahnson* 416

The Measurement of Bleeding Volume in the Dog for Studies on Blood Substitutes. *Hampden Lawson* 420

The Relative Value of Various Fluids in Replacement of Blood Lost by Hemorrhage, with Special Reference to the Value of Gelatin Solutions. *Hampden Lawson and W. S. Rehm* 431

The Effect of the Sodium Chloride Intake on the Work Performance of Man During Exposure to Dry Heat and Experimental Heat Exhaustion. *Henry Longstreet Taylor, Austin Henschel, Olaf Mickelsen and Ancel Keys* 439

Effects of Purified Antero-Pituitary Hormones on the Carbohydrate Stores of Hypophysectomized Rats. *V. V. Herring and Herbert M. Evans* 452

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THE EFFECT OF SALINE WASHINGS OF ISOLATED JEJUNAL LOOPS ON GASTRIC SECRETION[1]

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Received for publication June 19, 1943

Studies of the effects of implanting pedicle grafts of jejunum into the wall of the stomach have revealed that the normal responses to such secretory stimulants as histamine and alcohol of increased acidity of the gastric juice and lowered pH of the gastric mucosa may be reversed or markedly diminished (1–4). When portions of duodenum, jejunum, ileum or colon are used for such implants the factor causing this alteration of gastric secretion is seen to be confined to the duodenum and jejunum, the latter source possessing somewhat greater activity (3). Further studies have indicated that while a gastroenterostomy almost uniformly fails to produce this effect, it appears promptly after the bowel about the stoma is converted to a pedicle graft.

These findings have led us to postulate the presence in the lumen of the jejunum, and perhaps to a lesser extent in the duodenum, of a substance which, when introduced into the stomach, exerts a definite effect on the response of the acid secreting mechanism to certain secretogogues—notably histamine. The present report deals with the results of further efforts to demonstrate the presence of such a substance in isolated loops of jejunum.

METHODS. Healthy mongrel dogs (donors nos. 6–10, table 2) were operated upon and a segment of jejunum isolated as for a pedicle graft, but instead of its being opened and implanted in the wall of the stomach, one end of the segment was inverted under mattress sutures and the other end brought out through a stab wound at one side of the laparotomy incision. In three animals the segment was approximately 6 cm. in length, while in two it measured 12 to 15 cm. The continuity of the intestinal tract was re-established by end-to-end anastomosis.

After at least two weeks had elapsed to allow for healing, these loops were washed out two or three times with 25 cc. of normal saline and the washings collected.

To study the effects of such washings on gastric secretion the following technic

[1] This work was carried out under a grant from the John and Mary R. Markle Foundation.

was employed. Five normal dogs (recipients nos.1–5, table 2) which had had at least one previous gastric analysis and had been trained to tolerate a small stomach tube were used as subjects, having been without food for 24 hours. After collecting the normal fasting secretion during a period of 10 minutes, the entire 25 cc. of jejunal washings was instilled into the stomach and allowed to remain for 10 minutes when it was removed, together with the gastric secretion during this period. At this point the dog received the usual 1 mgm. of histamine

TABLE 1

Effect of washings of isolated jejunal loops on gastric secretion

Control series

Gastric analyses following histamine in normal animals

RECIPIENT NO.	FASTING SPECIMEN			0–10 MINUTES			10–20 MINUTES		
	Vol.	Free acid	Total acid	Vol.	Free acid	Total acid	Vol.	Free acid	Total acid
	cc.			*cc.*			*cc.*		
1. N	11	18	34	13	34	44	13	94	106
S	16	66	82	3.5	35	45	19	56	72
2. N	11	22	50	14	46	80	15.6	76	104
S	9	28	52	31	68	84	23	86	96
3. N	11	26	42	6	22	42	14	64	80
S	15	22	60	19	22	44	26	58	84
4. N	14	0	22	7	12	36	12	56	74
S	11	0	4	7	20	30	11	38	60
5. N	13	0	18	7	10	40	13	38	50
S	4	0	15	8	26	42	8	52	72
Averages	11.5	18.2	37.9	11.5	29.5	48.7	15.5	61.8	74.6

The following figures are derived from averages of animals

mgm. HCl	15.91	26.66	42.19
mgm. HCl/cc.	1.38	2.32	2.72
Parietal secretion	2.65	4.44	7.03
Nonparietal secretion	8.85	7.06	8.47

N = normal; S = saline.

phosphate intramuscularly and further specimens representing the secretory response over 10 minute periods were collected.

Two of the animals, nos. 2 and 3, received the washings from all five donor animals at different times, while the other three were used to test the material from one or more loops. In all, 16 tests were made in the five recipient animals.

The jejunal washings were found to be of pH 6.1 to 6.5 and to contain practically no titratable buffer. Thus, the pH was altered by 3 points when one drop of N/10 HCl or N/10 NaOH was added to 5 cc. of the washings.

As further controls, each of the five dogs used as recipients in the experiments were subjected to gastric analyses exactly comparable to those used to test the jejunal washings, except that saline alone was instilled.

RESULTS. *Effect on acidity of gastric juice.* While the specimen removed at the end of the 10 minute period during which the jejunal washings remained in the stomach always showed a marked reduction in the acidity, it seemed possible that the factor of dilution with an indeterminate portion of the 25 cc.

TABLE 2

Effect of washings of isolated jejunal loops on gastric secretion

Experimental series

GASTRIC ANALYSES					TIME AFTER HISTAMINE					
Animal		Fasting specimen			0–10 minutes			10–20 minutes		
Recipient no.	Donor no.	Vol.	Free acid	Total acid	Vol.	Free acid	Total acid	Vol.	Free acid	Total acid
		cc.			*cc.*			*cc.*		
1	6	5	20	50	7	12	22	8	92	110
	7	15	50	66	3	10	20	18	36	56
2	6	6	56	68	5	10	20	7	38	56
	8	9	30	58	24	5	16	16	10	26
	7	2	0	10	5	8	28	21	52	66
	9	16	70	88	24	14	30	33	66	88
	10	8	54	72	14	12	26	19	48	74
3	8	32	32	54	40	8	28	18	24	52
	7	33	20	44	5.5	0	20	14	30	54
	10	13	50	70	8	6	22	26	42	62
	9	6	16	40	7	4	34	12	22	54
	6	8	48	80	17	6	28	10	10	36
4	7	5	0	4	7	0	8	10	44	70
	6	11	0	44	4	0	10	14	20	10
5	6	4	0	5	4	0	7	7	38	58
	7	7	5	25	11	10	30	13	30	60
Averages.....		11.2	28.1	48.6	11.6	6.5	21.8	15.3	37.6	52.5

The following figures are derived from averages of above animals

mgm. HCl.....	19.86	9.23	29.32
mgm. HCl/cc.....	1.77	0.79	1.92
Parietal secretion...	3.31	1.54	4.89
Nonparietal secretion.....	7.89	10.06	10.41

of material instilled might have influenced the result, and for comparison with the control series this specimen was therefore discarded.

The comparative results in the experimental and control series appear in the accompanying table. From these figures it is apparent that while the injection of 1 mgm. of histamine phosphate is followed by a considerable rise in both the free and total acidities in the control series, there is a definite fall in both

of these values when the injection follows the instillation into the stomach of washings of isolated jejunal loops. By the end of 20 minutes the effect of the instillation has worn off to some extent.

The changes noted in the free and total acidities are reflected in the amounts of HCl secreted and in the milligrams of acid per cubic centimeter of gastric juice, the concentration being affected to a greater extent than the volume. It is interesting therefore to calculate the secretion of the parietal cells according to the method used by Gray, Bradley and Ivy (5). This is based on the statements of Lui, Yuan and Lim (6) and of Hollander (7) that the secretion of the oxyntic cells contains approximately 6 mgm. of HCl per cubic centimeter. Thus, by dividing the total milligrams of acid secreted by 6, one obtains a figure representing the volume of "parietal secretion" and by subtracting this from the total volume, an index of the mucous and pyloric or the diluting secretion. While these figures cannot be considered as more than estimates, they do provide a rough gauge of the activity of these cells.

Reference to these figures suggests that the depression in the acidity of the gastric juice is the result of a corresponding diminution in the activity of the oxyntic cells.

The depression of the normal reaction of the gastric analysis to histamine in the experimental series only lends support to the idea that this is due to a specific factor which can be obtained by washing out the lumen of the isolated jejunum with normal saline.

SUMMARY

When 25 cc. of saline washings of isolated jejunal loops are instilled into the stomach of normal dogs and withdrawn after 10 minutes there follows a marked depression in the normal response of the gastric acidity to histamine which is maintained for at least 20 minutes, suggesting a substance within the jejunal lumen which is responsible for the effect.

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STUDIES ON THE EFFECT OF ANOXIC ANOXIA ON THE CENTRAL NERVOUS SYSTEM

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Although the effect of anoxia on the electroencephalogram (EEG) of the human is well established (Davis) no observations seem to have been reported in which in unanesthetized animals the effect of anoxia over a wide range of oxygen tensions has been investigated. Such a study appears to be desirable as a means of determining the relation between the severity of anoxia, the type of cortical potential and the general behavior of the animal. In the present study several modifying factors have also been investigated such as the rôle of the rate of ascent as well as drugs and dietary factors.

METHODS. The experiments were performed on more than 100 rats in which the EEG and, in some experiments, the pulse rates were recorded with an Offner ink crystograph. The rats were placed in glass jars of 1 gallon capacity and the barometric pressure was lowered according to table 1 by means of a Cenco vacuum pump. For further details compare Kessler and Gellhorn.

RESULTS. I. *The effect of "moderate" anoxia on the EEG.* Three series of experiments (cf. A., B., E. in table 1) were performed in which the effect of lowering the barometric pressure to 190 mm. Hg was studied on the EEG. Figures 1 and 2 illustrate the typical results obtained under these conditions on 38 rats. Figure 1 shows that shortly after the level of 190 mm. Hg has been reached the electrical potentials disappear almost completely. At this time the animal appears to be unconscious, does not react to any stimuli and is unable to right itself. This latter observation seems to indicate not only that cortical activity is practically eliminated as seen by the EEG record but also that the functions of the pons are likewise greatly depressed. This period of temporary silence occurs, as table 2 indicates, in 76 per cent of all experiments. In spite of the fact that the rat remains exposed to this lowered oxygen tension, there is a gradual resumption of electrical activity as illustrated in figure 1. Alpha potentials reappear and later periods of very large potentials of a frequency of 8 to 10 per second are observed. These spindles show an amplitude of 100 μv or more and continue to be present at this barometric pressure until the end of the experiment (30 min.). Although spindles occur in practically all experiments of this type, their onset is somewhat variable. They appear earlier in those cases in which the sudden lowering of the barometric pressure does not lead to a period of complete cortical silence.

Figure 2 shows a record which is representative of a minority of experiments performed under the same conditions. In these cases no period of complete

[1] Aided by a grant from the Josiah Macy, Jr. Foundation.

cortical silence is observed but instead large delta waves appear. They decrease gradually in amplitude and then records are obtained in which spindles of a frequency of 8 to 10 alternate with small potentials similar to those seen at normal oxygen tension (fig. 2). Both types of observations have in common the fact that the rapid ascent leads at first to a more severe depression of cortical activity than is observed later after some adjustment reactions have taken place. When rapid, small potentials alternate with spindles, the rats are still markedly depressed but the righting reflex has been restored.

TABLE 1

Procedure of decreasing barometric pressure

TIME	PRESSURE				
	A	B	C	D	E
minutes	*mm.* Hg	*mm.* Hg	*mm.* Hg	*mm.* Hg	*mm.* Hg
1	580	580	580	580	580
2	480	480	480	480	550
3	380	380	380	380	500
4	280	280	280	280	450
5	190	190	190	190	400
6					350
6–10				190	
6–15		190			
6–36	190		190		
7–10					300
11–15					280
11–16				160	
16–20					260
16–46		160			
17–22				140	
21–25					240
23–28				120	
26–30					220
29–39				110	
31–60					190
37–67			140		

In order to determine the cause of the disappearance of the silent period or of the period of large delta waves which occur early during the anoxia, blood pressure records would be of greatest importance. Unfortunately, such records could not be obtained for the present set of experiments but the pulse rate was recorded (table 2). The records showed that the pulse rate was regular at that time and not essentially different from that of the control values obtained at 750 mm. Hg or from the rate observed after recovery from the silent period had been achieved.

In spite of it, it appears to us rather probable, unless blood pressure experiments would prove the contrary, that the restoration of cortical potentials is due to an improvement in the oxygenation of the brain rather than to an adaption of the cortical cells to a low oxygen tension.

If the barometric pressure is lowered from 190 mm. Hg to 160 mm. Hg after rats have been exposed to the former for 10 minutes (cf. column B in table 1) it is found that spindles also occur at 160 mm. Hg. They are likewise observed in experiments in which the level of 190 mm. Hg is reached very gradually (cf. column E of table 1). Under these conditions, spindles are not preceded by either a period of complete silence or delta waves (table 2). If the barometric

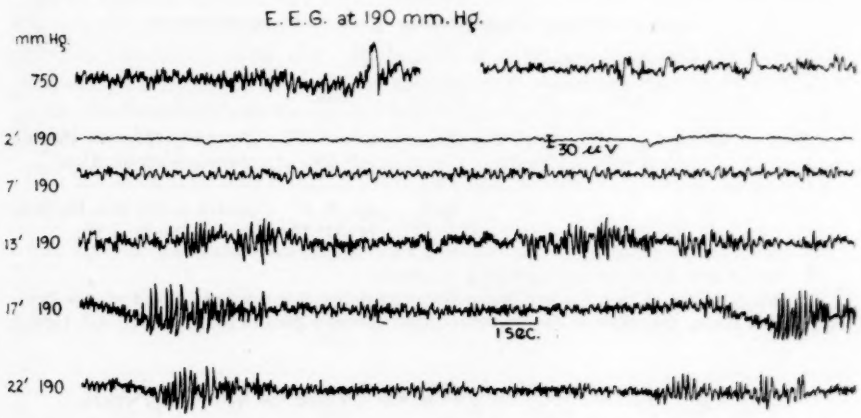

Fig. 1. The effect of reduced barometric pressure on the electroencephalogram of the unanesthetized rat. The pressure was reduced from 750 mm. Hg to 190 mm. Hg in five minutes (cf. table 1A).

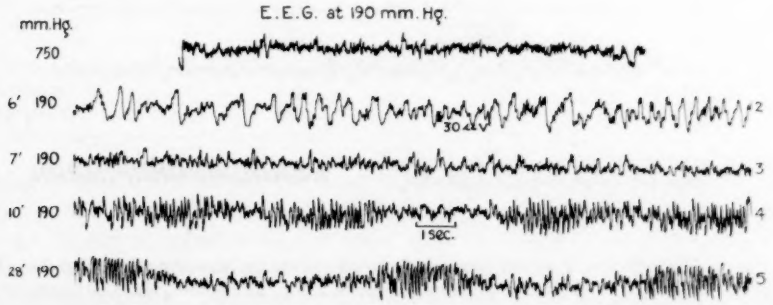

Fig. 2. The effect of reduced barometric pressure on the electroencephalogram. Procedure as in figure 1.

pressure is lowered more rapidly to levels below 190, spindles do not occur at 190 or 160 mm. Hg. The experiments suggest that spindles are characteristic of a certain range of oxygen tensions since they were almost never observed at a barometric pressure above 400 mm. Hg and were likewise absent at pressures of 140 mm. Hg or below. Another condition for their appearance is a time factor inasmuch as very rapid ascent through the indicated range does not lead to spindle formation.

II. *The effect of "severe" anoxia on the EEG.*[2] Two procedures (cf. C and D in table 1) were used in order to lower the barometric pressures below 160 mm. Hg. In procedure C a level of 140 mm. was reached in 36 minutes. Figure 3 shows that the lowering of the barometric pressure to 140 mm. Hg abolished the spindles seen at 200 mm. Hg. All potentials gradually decline in amplitude and

TABLE 2

Effect of decreased oxygen tension on the electroencephalogram

NO. OF RATS	PERIOD OF CORTICAL SILENCE			SPINDLES		REMARKS
	Per cent	Onset	Duration	Per cent	Onset	
38	76	Av. 1.6′ (1′–5′)	Av. 4.4′ (1′–7′)	95	Av. 16.3′* (7′–23′)	Ascent to 190 mm. Hg in 5 min. (Method A)
12	0		0	92.5	Av. 21.2′ (4′–37′)**	Ascent to 190 mm. Hg in 30 min. (Method E)

* Time in minutes from the beginning of ascent.

** One rat showed spindles 4 minutes after beginning of ascent at which time the pressure was 450 mm. Hg; the rest developed spindles between 280 and 200 mm. Hg. Cf. table 1

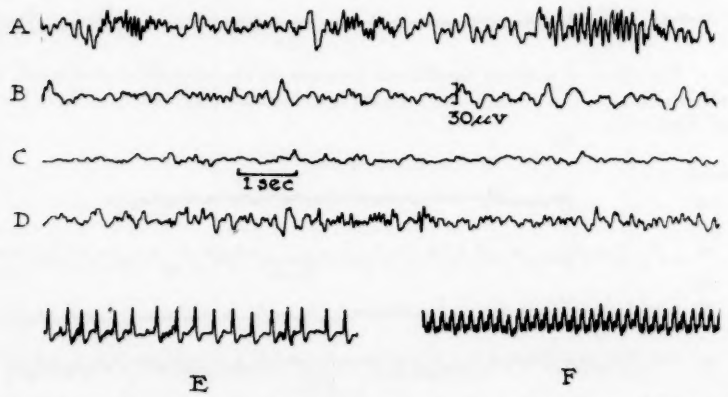

Fig. 3. The effect of lowered barometric pressure on the electroencephalogram. Procedure C of table 1. A, 13 min. at 200 mm. Hg; B, 2 min. at 140 mm. Hg; C, 12 min. at 140 mm. Hg; gasping; D, 10 min. after readmission of air; E, pulse rate taken during record C; F, pulse rate taken during record D.

become slower. Gasping occurs in most instances within 30 minutes (average 20 min.). At that time only few and slow potentials are seen. If the barometric pressure is not immediately increased, the animal dies. The occurrence of slow gasps indicates that under these conditions not only cortex and pons are

[2] The term "severe" anoxia has been used in this paper to denote the involvement of the medullary centers.

greatly depressed, since cortical potentials are practically absent and righting reflexes are abolished, but that the medulla oblongata is likewise affected (Lumsden). At the same time the recording of the pulse rate indicates gross irregularities and slowing (cf. record E in fig. 3). Readmission of air restores cortical potential, normal behavior, and regularity of the pulse (cf. records D and F in fig. 3).

In 11 animals the barometric pressure was lowered rather rapidly to 110 mm. Hg (procedure D of table 1). Of the 11 animals, 10 gasped at a pressure varying between 140 and 110 mm. Hg. Here again gasping was accompanied by a slow irregular pulse, absence of cortical potentials and loss of righting reflexes.

III. *Factors modifying the effect of lowered barometric pressure on the EEG.* In fifteen experiments, the effect of benzedrine (5 mgm./kgm., intraperitoneally) was studied on the EEG of rats subjected to procedure A. It was found that a

TABLE 3

Effect of decreased barometric pressure (190 mm. Hg.—Method A) on pulse rate

RAT	PULSE RATE PER MINUTE		
	Control	190 mm. Hg	
		Cortical silence onset	Recovery onset
1	420	400	388
2	390	412	410
3	374	378	388
4	366	313	340
5	532	456	500
6	480	420	434
7	400	448	450
8	458	384	382
Average	428	401	412
Per cent	100	94	96

period of silence was observed in 9 out of 15 experiments (60 per cent), a figure which is not essentially different from that seen in uninjected controls (76 per cent). However, spindles did not occur after the recovery from the period of silence in any of these animals (fig. 4) whereas they were found in 95 per cent of the control animals. At the same time it was observed that the rats, instead of being depressed as uninjected controls were at 190 mm. Hg. during the time when spindles occurred, appeared entirely normal: they moved about in the low pressure chamber and reacted to external stimuli. If benzedrine injected rats were subjected to procedure B in which the pressure was lowered to 160 mm. Hg, the EEG failed again to show spindles which regularly occurred in the corresponding control experiments.

It is important to note that this fundamental difference in the reactivity of the cortex existing between "benzedrine" and control rats is limited to the range of oxygen tensions of 160 to 200 mm. Hg since entirely different results were ob-

tained under conditions of "severe" anoxia. Benzedrine rats which were subjected to procedure D showed in all nine experiments that gasping occurred between 140 mm. Hg and 110 mm. Hg at times similar to those observed under control conditions; moreover, experimental and control rats showed a similar behavior and similar changes in EEG. These experiments suggest that observations made on animals subjected to a moderate degree of anoxia may not permit

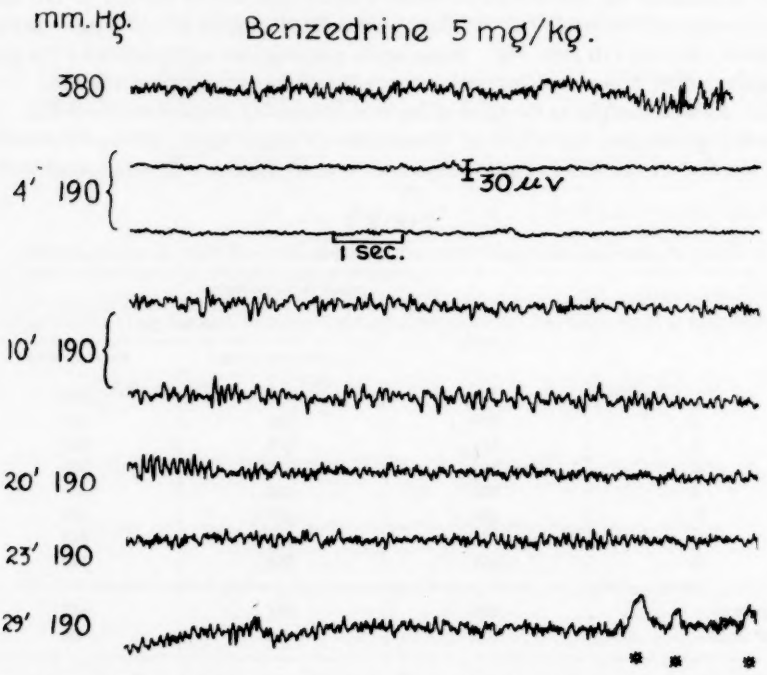

Fig. 4. The effect of benzedrine (5 mgm./kgm.) on the electroencephalogram of the unanesthetized rat under conditions of lowered barometric pressure. Asterisks indicate movements.

one to make any prediction with regard to their behavior at extreme degrees of anoxia and vice versa.

In a number of investigations the resistance of animals to anoxia under various conditions has been tested by determining their survival time under conditions of extreme anoxia. Thus Campbell found that carrot-fed rats show an increased resistance to anoxia. We were able to confirm this finding on fifteen rats since only six rats (40 per cent) gasped within 10 minutes at 100 mm. Hg, whereas in rats fed dog chow diet, ten of eleven rats (91 per cent) gasped within this period. On the other hand, it was shown that this increased resistance displayed by the carrot-fed rats under conditions of "severe" anoxia did not appear when the

animals were subjected to "moderate" anoxia (procedure A of table 1). Under these conditions behavior and EEG were similar and apparently independent of the diet.

Discussion. With respect to the reversible period of electrical silence of the cortex which occurs, after rapid ascent, at a barometric pressure of about 190 mm. Hg it was found that in spite of continued anoxia, the brain potentials gradually recover to the "spindle" type. The absence of changes in pulse rate when recovery from this period of temporary cortical silence occurs suggests that systemic circulatory changes are not involved in the recovery. It is, however, not improbable that an improved oxygenation of the cortex is the cause of this recovery, since dilatation of cerebral vessels may gradually increase in anoxia particularly if acid metabolites are accumulated at the same time.

The period of electrical silence of the cortex observed at a barometric pressure of 190 mm. Hg is fundamentally different from that observed at pressures of 140 mm. and below since the latter ends invariably in death unless the barometric pressure is quickly raised. The reversible form of electrical silence (at 190 mm. Hg) occurs under conditions indicating an anoxic involvement of cortex and brain stem down to the pontine level (righting reflexes). The irreversible form of cortical silence (at 140 mm. Hg) is accompanied by an involvement of the whole brain including the medulla oblongata as indicated by gasping, slowing and irregularities of pulse rate suggesting vagal discharges. If the barometric pressure is restored rapidly, the animals may completely recover. The observations made at 190 mm. Hg on one hand and at 110 to 140 mm. Hg on the other hand give renewed evidence for the well established fact that anoxia affects more easily cortex and brain stem than medullary centers (cf. Gellhorn, 1943, p. 166). They do not support Windle's claim that anoxia results in fortuitous damage to various parts of the central nervous system.

The spindles observed at 190 mm. Hg are similar to those reported by Sugar and Gerard following recovery from complete anemia of the brain and interpreted by them as an indication of increased excitability of the cortex. Since their experiments were carried out in narcotized animals, no comparison with the general behavior could be made. It is, therefore, of interest to emphasize that our observations clearly showed a marked depression of the animals during the period in which trains of spindles prevailed. Whereas the animals showed no spontaneous movements at 190 mm. Hg and appeared to have also a diminished tone of the muscles, they assumed normal activity and behavior as soon as the pressure was raised to a level at which these spindles disappeared.

Our experiments have confirmed Campbell's findings but have shown that in spite of the greater resistance of carrot fed animals to lethal tensions of oxygen, they appear not to be more resistant to milder degrees of anoxia inasmuch as they show the same cortical and clinical changes as do control rats. Similarly, it has been found that benzedrine which decidedly improves the performance of rats at 190 mm. Hg does not in any way alter their resistance to lethal tensions of oxygen. It must be concluded that experiments done under conditions using

the time of survival under severe anoxia as the criterion of anti-anoxic properties of certain drugs or procedures are more of pharmacological than physiological interest.

SUMMARY

Studies on the effect of lowered barometric pressure on unanesthetized rats have shown that when the ascent is carried out rapidly a period of temporary silence of the cortex occurs at a level of 190 mm. Hg from which the animals recover spontaneously in spite of continued lowered barometric pressure. During the period of cortical silence righting reflexes are absent. Recovery is accompanied by the appearance of large spindles of a frequency of eight to ten per second. Righting reflexes reappear during this period but the animals remain depressed. Rats injected with benzedrine show similar changes in EEG as far as the period of cortical silence is concerned, but they show during the subsequent recovery a normal EEG without spindles and without any other signs of depression of activity. If the pressure is gradually lowered to 190 mm. Hg, the periods of temporary silence may be absent, but spindles accompanied by a general depression of the animals are found. The occurrence of spindles is confined mostly to barometric pressures varying between 280 mm. Hg and 160 mm. Hg. If the pressure is lowered to 110 to 140 mm. Hg, a period of electrical silence occurs from which no recovery at that barometric pressure is possible. As the gasping and slowing of pulse show, the medulla is involved under these conditions.

Benzedrine which increases the resistance of rats to anoxia as indicated by the EEG at moderate degrees of anoxia does not increase the resistance or the survival time of rats under conditions of extreme anoxia. Conversely, rats fed with carrots although showing an increased survival time under lethal conditions (Campbell) do not show any improvement in performance or in EEG under conditions of more moderate anoxia.

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THE EFFECT OF ANOXIC AND ANEMIC ANOXIA ON THE LEUCOCYTE COUNT

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In a previous investigation it was shown by the present authors that chemically (metrazol) and electrically induced convulsions call forth a neutrophilic leucocytosis in normal animals. However, no changes in the leucocyte count occurred in adreno-demedullated rats subjected to the same measures. These data indicate that leucocytosis may be the result of an excitation of centers of the sympathetico-adrenal system leading to a discharge of adrenalin which by direct action on the bone marrow results in an increased discharge of neutrophilic leucocytes. In addition to this mechanism other mechanisms are involved also which appear to be independent of the discharge of adrenalin. It was found that the injection of typhoid-paratyphoid vaccine led to a neutrophilic leucocytosis which was not confined to the normal animals but was also present in adreno-demedullated rats to a similar degree (Clare, Cress and Gellhorn, 1943).

Extensive studies (cf. Gellhorn, 1943, for literature) have shown that anoxia causes an excitation of the sympathetico-adrenal system. It is likewise known that anoxia, at least in its earlier stages, is accompanied by a leucocytosis later followed by leucopenia (Meyer, Seevers and Beatty, 1935; Ruppanner, 1920). The question, therefore, arises as to whether anoxia causes leucocytosis by means of sympathetico-adrenal discharges. Since it is known that not only anoxic anoxia but also anemic anoxia (Nasmith and Graham, 1906) call forth leucocytosis as well as excitation of the sympathetico-adrenal system, it was decided to investigate the dependence of leucocytosis on the sympathetico-adrenal system under these conditions.

METHOD. The experiments were performed on 52 rats averaging 250 grams in weight. Half of the animals were adreno-demedullated. Blood samples were obtained from the tail after amputation of 1 or 2 mm. and discarding of 2 or 3 drops. Control experiments reported in our previous work showed only insignificant changes in leucocyte count taken at two hour intervals over 24 hrs. Operated animals were used not earlier than 3 to 4 weeks after operation. No food was given during the periods of anoxia.

Anoxic anoxia was established by subjecting the animals to a barometric pressure of 400 mm. Hg in a low pressure chamber for 12 hrs. on each of four successive days. Before and after the period of anoxia red and white counts were taken. The effect of anemic anoxia was studied in two ways: 1st, by subjecting rats to carbon monoxide poisoning, and 2nd, by hemorrhage. Carbon monoxide was found to be effective when injected intraperitoneally (1 cc. per 20 grams' body weight). Blood counts were taken before, and 6 and 12 hrs. after the injection.

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In order to study the effect of hemorrhage, about ⅓ of the total blood was removed from the tail in 30 minutes. It was assumed that the rat has 6.7 cc. of blood per 100 gram of body weight (Griffith and Ferris, 1942). Blood counts were taken before bleeding and 13, 17 and 24 hrs. after bleeding. A differential count was carried out on 10 normal rats subjected to anoxic anoxia and CO poisoning.

RESULTS. The effect of anoxic anoxia on 5 of the 10 animals which were investigated is shown in table 2. It is clearly evident that anoxic anoxia causes a decided increase in leucocytes in normal but not in adreno-demedullated rats. The leucocytosis occurs in most instances at the end of the first period of anoxia; only in two out of ten animals was the leucocytosis at its maximum after the second anoxic period. Thereafter, there was a gradual decline in leucocyte count so that at the end of the fourth anoxic period the count was on the average only slightly above the control levels. The effect is not due to hemoconcentration as evidenced by the fact that the changes in erythrocytes during these experiments

TABLE 1

Effect of various forms of anoxia on the white cell count

NO. OF RATS	AVERAGE WHITE COUNT IN MM³ (CONTROL)	ANOXIA	AVERAGE WHITE COUNT IN MM³ (AFTER ANOXIA)	INCREASE IN % OF CONTROL	PERCENTAGE before anoxia		PERCENTAGE after anoxia		PERCENTAGE INCREASE IN NEUTROPHILS
					Neutrophils	Lymphocytes	Neutrophils	Lymphocytes	
5	21080	CO 1 cc./20 grams weight intraperitoneally	28860	37	30	70	62.5	37.5	108
5	14550	16 hrs. at 300 mm. Hg	18000	24	26.8	73.2	43.2	56.8	61

were only very slight. After the end of the first anoxic period the leucocytes in 10 rats had increased from 18,000 to 30,000 per mm³ whereas the red counts increased from 9.4 to 9.7 millions per mm³.

None of the adreno-demedullated rats showed a leucocytosis. In two adreno-demedullated rats (nos. 5 and 8; the record of the latter is not shown in this paper) there was a decided fall in leucocytes but in most observations no significant effects were observed. Whereas the maximal increase in white count of 10 normal rats averaged 82 per cent, it amounted to 4.7 per cent only following anoxia in 10 adreno-demedullated rats.

The influence of severe hemorrhage on the blood count on normal and adreno-demedullated rats is illustrated by table 3. Here again it is shown that leucocytosis is confined to the normal group and appears at its height 8 to 12 hrs. after bleeding. Hereafter, the white count gradually returns to control levels. For the interpretation of these findings, the curve illustrating the red count is important. It shows that four hours following the bleeding, the red count is reduced to approximately 50 per cent of the original value and remains at this

TABLE 2

Effect of low barometric pressure (12 hrs. at 400 mm. Hg) on the leucocyte count

HOURS	PROCEDURES	A. NORMAL RATS										B. ADRENODEMEDULLATED RATS									
		#1		#2		#3		#4		#5		#1		#2		#3		#4		#5	
		E*	L†	E	L	E	L	E	L	E	L	E	L	E	L	E	L	E	L	E	L
0	Control	9.6	15.6	9.0	13.5	9.1	15.0	9.3	20.0	8.9	18.0	9.7	28.0	8.9	19.0	8.5	19.5	9.3	18.0	8.0	24.0
	Counts		16.0		14.0		14.5		21.0		17.8		28.5		19.5		19.0		19.0		24.4
1–13	Anoxia																				
13	Counts	9.5	16.0	9.4	34.0	9.2	27.0	9.5	32.5	9.1	39.2	10.4	30.5	9.4	19.4		18.0	9.6	18.6	7.8	22.0
13–25	Rest																				
25–37	Anoxia																				
37	Counts	10.4	23.5	9.6	26.0	10.0	25.0	9.3	21.0	10.0	26.0	10.3	31.0	9.1	18.5		19.5	9.5	18.3	6.8	25.0
37–49	Rest																				
49–61	Anoxia																				
61	Counts	10.5	21.0	9.5	24.0	10.1	21.0	9.7	22.0	10.1	23.0	9.8	28.0	11.0	23.0		19.5	9.4	20.0	8.0	17.0
61–73	Rest																				
73–85	Anoxia																				
85	Counts	10.4	19.0	9.5	21.0	9.8	18.0	9.5	20.5	10.0	20.0	9.0	29.0	10.0	16.0		19.0	9.6	18.5	8.2	19.2
L maximal increase (%).....			48.7		146.1		82.5		58.5		118.9		9.5		19.2		1.0		8.1		3.3

Average maximal increase of L in 10 normal rats = 81.7%. Average maximal increase of L in 10 adrenodemedullated rats = 4.8%.

* E = Erythrocytes in millions per cu. mm.

† L = Leucocytes in thousands per cu. mm.

TABLE 3

Bleeding experiments

RAT	WGT.	CONTROL E	CONTROL I L	CONTROL II L	CC. BLOOD REMOVED	LEUCOCYTES						% INCR. IN L	ERYTHROCYTES					
						3 hrs.	7 hrs.	11 hrs.	13 hrs.	17 hrs.	24 hrs.		3 hrs.	7 hrs.	11 hrs.	13 hrs.	17 hrs.	24 hrs.
						After bleeding							After bleeding					
Normal rats																		
	gms.																	
1	210	9.0	18.5	16.0	4.0	15.8	21.2	31.3	25.7	23.0	19.2	81.0	5.5	3.9	4.2	4.0	4.5	4.8
2	200	9.0	18.5	17.0	4.0	19.0	13.7	24.8	22.4	16.5	20.0	39.2	5.4	3.8	4.2	4.6	4.5	5.0
3	220	8.5	20.0	21.0	4.5	16.0	40.0	27.0	32.5	19.5	29.5	99.1	5.1	5.2	5.0	5.4	5.5	5.2
4	230	10.0	20.0	22.0	4.5	26.0	35.0	29.8	25.0	23.5	24.0	66.6	5.1	4.1	4.0	4.2	4.5	4.4
5	240	8.9	20.0	19.0	5.5	16.3	30.0	35.0	27.5	24.0	19.2	79.5	5.0	5.7	5.2			5.0
6	280	9.0	22.3	22.0	5.5	22.5	39.0	28.0				75.1	5.8	5.5	5.9			
Average												73.4						
Adreno-demedullated rats																		
1	225	9.8	30.0	29.0	4.5	20.4	19.4	20.1	20.6	22.0	22.6	−34.1	5.8					6.0
2	275	9.7	19.0	18.9	5.5	17.0	20.0	17.3	18.0	20.0	21.2	11.6	5.6	5.2	5.3	5.0	5.2	5.3
3	295	9.6	20.3	19.0	5.5	17.5	13.0	18.0	17.8	16.8	16.0	−33.6	5.9					4.7
4	235	8.45	27.0	26.0	5.0	20.0	24.0	25.0				−24.6	6.2	5.0	6.0			
5	240	9.8	28.0	27.0	5.0	15.0	22.0	24.0				−20.0	6.0	5.2	5.9			
6	165	8.9	22.0	23.0	5.0	24.0	22.5	23.0				6.7	5.6	4.9	5.0			
Average												−15.7						

level for the next 24 hours indicating that the blood volume has increased considerably. In spite of this dilution, the increase in leucocytes averages 72 per cent in six normal rats. The actual production and release of leucocytes into the circulatory system must have been considerably greater than is indicated by this figure.

The adreno-demedullated rats show in 4 out of 6 animals a decrease in white count after bleeding which roughly parallels the decrease in red count and may

TABLE 4

Effect of CO on the leucocyte count*

RAT	CONTROL E	CONTROL I L	CONTROL II L	LEUCOCYTES 2 hrs. after CO	LEUCOCYTES 6 hrs. after CO	LEUCOCYTES 12 hrs. after CO	% INCR. L	E 6 HRS. AFTER CO	E 12 HRS. AFTER CO
				Normal rats					
1	8.8	21.0	20.0	17.5	28.2	27.0	37.5	8.0	8.0
2	9.0	20.0	20.0	15.0	27.8	24.5	39.0	8.5	8.6
3	9.0	30.0	29.7	21.8	40.0	37.0	33.3	9.1	9.0
4	9.5	17.5	16.0		32.0	28.0	91.6		
5	9.0	20.7	20.0		32.0	25.0	56.8		
6		21.0	18.5		30.0	22.0	51.5		
7		19.8	16.0		31.0	24.0	73.1		
8		23.2	20.0		21.0	36.0	66.6		
9		17.4	17.0		15.2	33.5	94.7		
10	9.4	25.0	23.0		36.5	32.0	52.1	9.0	
Average							54.4		
				Adrenodemedullated rats					
1		23.6	22.0	27.6	27.4	25.0	20.1		
2		32.2	31.0	31.0	32.0	29.0	1.3		
3	8.7	28.0	27.0	27.0	26.0	26.5	−5.0	8.9	9.0
4	8.9	30.0	28.0		31.0	31.5	8.6	9.0	8.8
5	8.9	20.0	19.0		23.0	22.0	17.9	8.9	
6	9.3	19.0	18.5		21.0	21.0	11.7	9.0	
7	9.8	22.4	22.3		23.0	21.0	2.7	9.2	
8	8.5	28.0	27.0		27.5	27.0	−1.9	8.4	
9	9.0	29.0	28.0		28.0	29.0	1.8	9.3	
10	9.4	25.6	24.0		26.0	24.0	4.9	9.5	
Average							7.6		

* 1 cc. CO per 15 to 20 grams weight, intraperitoneally.

be accounted for by the dilution of the blood. In two rats the leucocytes show an insignificant rise (7 and 12 per cent respectively).

Normal rats under carbon monoxide poisoning show an increase in the number of leucocytes which is maximal after 7 or 13 hrs. (table 4). It amounts to 60 per cent on the average whereas the average change in the demedullated group is 6 per cent. After 24 or 48 hrs., the control value was obtained again in the unoperated group. These changes in leucocyte count were not accompanied

by any significant changes in the red blood count. Table 1 shows the leucocytosis which occurs in another group of normal rats under conditions of anoxic and anemic anoxia. Although the leucocytosis was of a lesser degree than in the main group of experiments described above, its neutrophilic character is unmistakable.

DISCUSSION. The experiments show conclusively that anemic and anoxic anoxia lead to a leucocytosis in normal but not in adreno-demedullated rats. The data suggest that the liberation of adrenalin in the normal group is the cause of the leucocytosis. This assumption is supported by the fact that anemic as well as anoxic anoxia leads to the liberation of adrenalin. That the results are not due to changes in blood volume is indicated by the simultaneous determination of the red count which does not show any significant changes in anoxic anoxia and in carbon monoxide poisoning in the present investigation. In the group showing anemic anoxia as a result of hemorrhage, there was a fall in red blood count indicating a marked dilution of the blood but this fall was similar in normal and adreno-demedullated rats. Nevertheless, only the normal group showed leucocytosis.

The experiments agree with our earlier studies in which metrazol and electroshock caused a leucocytosis which under these conditions was likewise confined to the normal and was absent in the adreno-demedullated group. Moreover, in both groups of experiments, it was found that the leucocytosis was neutrophilic only. Both investigations support the assumption that sympathetico-adrenal discharges are a potent factor in the elicitation of a neutrophilic leucocytosis. However, chemical substances may also have a direct peripheral effect on the bone marrow as shown by the fact that after injection of typhoid vaccine, leucocytosis appears in both normal and adreno-demedullated animals.

SUMMARY

The effect of anoxic and anemic anoxia is studied on the blood picture of normal and adreno-demedullated rats. It is found that anoxic anoxia induced by exposure of the animals to a barometric pressure of 400 mm. Hg for 12 hrs. on four successive days causes a leucocytosis after the first or second period of anoxia in normal but not in adreno-demedullated rats. Similarly, it is observed that carbon monoxide poisoning and hemorrhage are followed by a leucocytosis confined to the normal group. The leucocytosis is in all cases of a neutrophilic character. The experiments indicate that the leucocytosis in anoxic and anemic anoxia is due to a discharge of adrenalin following the excitation of the sympathetico-adrenal system. It is assumed that the liberated adrenalin acts directly on the bone marrow.

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THE EFFECTS OF POLYCYTHEMIA AND OF A CARROT DIET ON RESISTANCE TO ANOXIA

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Interest in measures designed to increase resistance to anoxia is understandably acute at the present time. Since one of the most constant physiologic reactions to anoxia is the increase in the number of red cells the possibility that an artificial polycythemia induced by blood transfusions might be beneficial to high altitude fliers merits investigation. Considering the ease of the operation and the long life of the red cell, such a method, if effective, might be quite practical. Authorities differ, however, on the importance of polycythemia as a factor in acclimatization as is evident on reading the chapter "Acclimatization" in *Anoxia, Its Effect on the Body*, by Van Liere, (1). Experimentally, Campbell (2, 3, 4) has studied the effects of an increased hemoglobin percentage on tissue oxygen tension, using the method of injected nitrogen bubbles, but we could find no reports concerning its effect upon actual resistance to anoxia.

In attacking this problem we were impressed by the great individual variation in the survival time of rats under conditions of acute anoxia. It also seemed to us that since the survival of high altitude personnel depended in large part upon the reception of sensory stimuli and the ability to respond to them, a test based upon the loss of a reflex response under anoxic conditions might be more appropriate than length of survival. Such a method was therefore developed and applied to the polycythemia problem.

Finally, the confirmation by Nelson, Goetzl, Robins and Ivy (5) of the original report of Campbell (6) that a ten day carrot diet markedly increased the resistance of rats to acute anoxia, raised the question whether such increased resistance would also be shown by our test.

EXPERIMENTAL. Albino rats of the University of Denver strain were used in these studies. The method employed was as follows. An electric bicycle horn was placed in the bottom of a vacuum desiccator, the leads sealed through the stopper and the horn operated by means of an outside switch. A wire frame, on which the animal rested, was placed over the horn so that he would not be affected by the mechanical vibration of the horn. Under these conditions, sounding the horn produced a uniform response, namely, a sharp, backward twitch of the ears, which we call the "Ear-batting Reflex," which is easily observed through the walls of the desiccator. Undue excitement is not produced, a rat lying quietly in the chamber will usually merely bat his ears when the horn is sounded.

Exposure to low barometric pressures results in loss of this reflex, after varying lengths of time. (We believe that failure of the animal to respond is probably due to its failure to hear the signal rather than to muscular paralysis, at least the

ability to respond to electrical stimulation persists much longer.) Disappearance of the reflex, when tested at 30 second intervals is usually unequivocal, that is, the last response may have been quite strong but 30 seconds later it will have

TABLE 1

Control reflex times, in minutes, for 5 determinations, made at weekly intervals

RAT NO.	1ST	2ND	3RD	4TH	5TH	AVERAGE	σ
17	14	10	7	6	10	9.4	2.8
4	14	14	12	13	14	13.4	.8
11	13	15	24	18	14	16.8	4.0
3	32	35	31	34	32	32.8	1.5
16	24	40	30	48	33	35.0	8.2

TABLE 2

Effects of polycythemia on reflex time

RAT NO.	BEFORE TREATMENT			TREATMENT*	AFTER TREATMENT					
					1 week		2 weeks		3 weeks	
	RBC†	Reflex time‡	σ		RBC	Reflex time	RBC	Reflex time	RBC	Reflex time
				cc.						
1	7.85	9.8	1.6	2.25	10.20	12	10.05	15	8.00	14
2	8.55	16.6	1.5	2.25	9.85	20	9.60	19	9.15	16
3	8.20	32.8	1.5	2.25	9.95	25	10.15	15	7.65	30
4	7.90	13.4	.8	3.2	11.60	16	10.40	14	7.40	14
5	9.0	22.0	3.5	3.3	10.60	19	10.10	25	9.10	22
6	8.35	24.6	2.8	3.15	9.65	13	9.60	22	9.00	28
Av.....	8.31	19.9			10.31	17.5	9.98	18.3	8.38	20.7
7	9.00	11.2	3.2	6.2	14.20	16	11.40	16	8.75	9
8	8.00	15.0	1.7	6.25	12.65	12	11.70	15	9.50	13
9	8.10	15.0	3.6	6.0	13.80	20	12.80	14	10.30	18
10	9.10	13.0	1.3	6.5	14.80	10	11.55	9	10.20	11
11	8.40	16.8	4.0	6.25	12.90	12	12.75	6	8.55	9
12	8.75	17.2	2.4	6.25	11.75	19	9.60	21	9.15	16
Av.....	8.56	14.7			13.35	14.8	11.63	13.5	9.41	12.7

* Red cells suspended in saline solution. The amounts given represent the volume of whole blood from which red cells were obtained.

† Red blood count in millions.

‡ Average of 5 determinations at weekly intervals before transfusion. Reflex time means the number of minutes elapsing between the time an altitude of 40,000 feet was reached and the time at which the reflex disappeared.

disappeared completely. Sometimes an animal failing to respond to the usual blast will respond if the signal is prolonged or repeated, but after another 30 seconds no response whatever can be elicited. Once lost, the reflex does not reappear unless the pressure is raised, whereupon it reappears promptly. It

therefore seems reasonable to assume that loss of the reflex is due to anoxia and the length of its survival measures the animal's resistance to this condition.

As regards variability, raw data on 5 of the rats used in this study are presented in table 1. These 5 rats represent the extremes of consistency and inconsistency and also the extremes of time of reflex survival. All other rats fall between these extremes, as shown by the standard deviations and average reflex times listed in tables 2 and 3. It will be noted that the variations in a given rat, determined from time to time, are less than the variations between different rats, which suggests the advisability, in future work, of using animals selected for their uniformity.

All determinations were made at a pressure of 140 mm., corresponding to an altitude of 40,000 feet, the rate of ascent being 2,000 feet per minute. The desired barometric pressure was obtained by means of a vacuum pump, air being admitted through an adjustable capillary. The circulation through the chamber at 40,000 feet was 4 liters of outside air per minute. After reaching

TABLE 3

Effect of carrot diet on reflex time

RAT NO.	AV'G. REFLEX TIME (5 DETERMINATIONS)	σ	REFLEX TIME, 10TH DAY	REFLEX TIME, 11TH DAY
13	21.8	3.2	19	18
14	10.0	1.8	13	19
15	17.6	1.5	17	21
16	35.0	8.2	56	45
17	9.4	2.8	12	11
Av'g.....	18.8		23.4	22.8

40,000 feet the horn was sounded at 30 second intervals and the period elapsing between reaching that altitude and the disappearance of the reflex, read to the nearest minutes, recorded as the "Reflex time."

The normal reflex time of 12 rats was determined by five tests made at weekly intervals. Their blood count was determined, following which they were injected, via the jugular vein, with rat red cells suspended in saline in the amounts, expressed in equivalents of whole blood, shown in the table. Tests were again made at weekly intervals for three weeks and after each test a red count was made. The results are shown in table 2.

To study the effects of a carrot diet 5 rats whose reflex times had been established as described were placed on such a diet for 11 days. The weight loss was 8.7 per cent. Their reflex times were again determined on the 10th and 11th days. These results are shown in table 3.

DISCUSSION. It will be noted that two levels of polycythemia were produced. In the first group of six rats (table 2), which received red cells equivalent to a blood transfusion of 2.25 to 3.3 cc. each, the increase in red count was 24 per cent, while in the second group, receiving from 6.0 to 6.5 cc. the increase was 56

per cent. This was done to take into account the possibility that the advantage of a high red count might be overbalanced by the greater blood viscosity. At neither level, however, was there an increased resistance to anoxia. This agrees with the conclusion reached by Campbell (4) that an increased amount of hemoglobin at high altitudes is of no value.

As regards the carrot diet, the increase in resistance shown is slight and statistically insignificant. Although, using the percent of animals surviving acute anoxia as the criterion, Ivy's work (6) is conclusive in proving beneficial effects, this diet would appear to have little value under conditions where survival depended upon reflex responses, insofar, at least, as the response used in this study may be regarded as representative.

CONCLUSIONS

1. A method of studying anoxia in the rat, based on the loss of a reflex response to sound, is described.

2. Using this method, no increase in resistance to anoxia was produced either by a moderate (24 per cent) nor high (56 per cent) degree of polycythemia.

3. A 10 day diet of carrots exclusively was also ineffective.

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WATER AND FAT CONTENT OF ORBITAL TISSUES OF GUINEA PIGS WITH EXPERIMENTAL EXOPHTHALMOS PRODUCED BY EXTRACTS OF THE ANTERIOR PITUITARY GLAND[1]

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When anterior pituitary gland extracts are injected into thyroidectomized guinea pigs, an exophthalmos and hypertrophy of the retrobulbar fat and extraocular muscles is produced (1, 7, 9). Study of histological sections of the orbital fat and muscles of such animals have shown that there is an increase in the quantity of interstitial water in these tissues. It has been impossible to determine, however, whether the lipid and the connective tissue constituents were increased in amount. For this reason quantitative determinations were made of the water, lipid (ether soluble) and connective tissue components of the orbital fat, and of the water content of the extraocular muscles of 87 exophthalmic and control female guinea pigs. The water content of peritoneal depot fat and of skeletal muscle of 70 of these animals was also determined.

MATERIALS. A marked exophthalmos was produced in 19 thyroidectomized guinea pigs by the injection of an extract of anterior pituitary glands. In addition, 17 normal animals and 26 thyroidectomized but uninjected guinea pigs were used as controls. An analysis was also made of the orbital tissues of nine animals in which injection of the hypophyseal extract failed to produce an exophthalmos or in which the proptosis had been transitory and had disappeared at the time of autopsy. In order to provide a control for the extract used, 10 thyroidectomized guinea pigs were injected with extracts of either heart, kidney, or spleen. These extracts were identical in method of preparation and administration with those of the hypophyseal tissue. Six uninjected thyroidectomized guinea pigs were added to the group which were injected with the control extracts.

The extract used to produce an exophthalmos was prepared from the anterior lobe of beef pituitary glands by the method described earlier (10). Fifteen to 20 mgm. of this preparation were injected subcutaneously per day for 60 to 70 days. All of the injected animals were thyroidectomized 10 to 20 days prior to the first injection. The post-thyroidectomy period of the uninjected control animals was the same as that of the guinea pigs injected with pituitary gland extract.

The average and range of body weights of the animals in the experimental and control groups were strictly comparable with the exception of those guinea pigs to which control extracts were given.

METHODS. Since the amount of orbital muscle and fat in the guinea pig

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is very small, the methods used to determine their lipid and water content necessarily differed from standard procedures. The animals were killed with illuminating gas, immediately decapitated, and bled so that extremely little or no blood remained in the orbital vessels. The orbital fat and the extraocular muscles, including the levator, were removed and placed in small glass stoppered weighing bottles. The fat, after any excess fluid had been absorbed with filter paper, was rapidly spread over the bottom of the bottle in a thin layer. The muscles were scattered singly on the walls of the bottle. The bottles and their contents were immediately weighed and then dried *in vacuo* over phosphorus pentoxide at 40°C. until the weights were constant (±0.1 mgm.). After the drying of the orbital fat the lipid was extracted with six or seven changes of anhydrous ether. The residue was then dried, weighed, and the extractions repeated. Due to the toughness of the fat no fragments were dislodged and lost in the ether extraction. Following the second series of extractions, the residue was dried to a constant weight and the loss in weight caused by the removal of the ether soluble material was taken as the weight of the lipid content of the fat. The largest component of the residuum was connective tissue.

Since the amount of fat and muscle available from a single orbit was very small, duplicate analyses were impossible. However, determinations of the water and lipid content of the tissues from the right and left orbits of the same animals were found to agree closely, so that the methods used are believed to be adequate.

In order to discover whether the changes induced by the injection of anterior pituitary extracts were limited to the orbital tissues, the water content of the peritoneal fat and of skeletal muscle was determined. The fat used in these experiments was taken from the dorsal aspects of the peritoneal cavity, around the adrenals, kidney, ureters and from the uterine mesentery. The mass of peritoneal fat was spread out thinly in a Petri dish, weighed, and then dried in the oven at 100°C. Approximately 20 grams of muscle, including most of the musculature of a fore and hind limb, the abdominal wall, and the psoas and scalene muscles were used. These muscles were rapidly freed of surrounding fat and cut up coarsely with scissors. They were weighed in a Petri dish and dried at 100°C. until the weight was constant (±25 mgm).

The degree of exophthalmos was determined, in addition to inspection and palpation of the globe in the living animal, by measuring, at autopsy, the protrusion of the globe from the orbit. After the head had been skinned and severed from the body, the distance from the limbus corneae to the edge of the bony orbit, at the supra-orbital notch, was measured with vernier calipers (the skull-limbus measurement).

RESULTS. *Orbital tissues.* The injection of extracts of the anterior pituitary glands produced a marked exophthalmos, as determined by the increase in the skull-limbus measurement from 3.8 mm. in the thyroidectomized control animals to 5.6 mm. in the exophthalmic animals (table 1)[2]. The weight of the orbital

[2] Ablation of the thyroid gland caused a slight increase (0.6 mm.) in the skull-limbus measurement. This effect has been consistent and is more marked in guinea pigs thyroidectomized for very long periods.

fat and muscles of the latter was increased 115 per cent ($P < 0.01$)[3] and 33 per cent ($P < 0.01$) respectively. Data from the control series show that thyroidectomy or injection of the control extracts had no effect on the weight of the orbital fat or on that of the extraocular muscles. There was, however, an increase of 39

TABLE 1

Comparison of water and lipid content of the orbital tissues of control and exophthalmic guinea pigs

NUMBER OF CASES	SKULL-LIMBUS	BODY	ORBITAL FAT				EXTRA-OCULAR MUSCLES			
			Total	Water	Lipid	Residue	Total	Water		Residue
A. Normal control group										
	mm.	*grams*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	%	*mgm.*
17	3.2	546	43.1	24.8	14.9	3.3	78.8	63.4	80.5	15.3
ϵ_M	0.09	23	2.5	1.6	1.6	0.3	2.2	1.7	0.2	0.6
B. Thyroidectomized, uninjected control group										
26	3.8	529	47.2	29.4	14.1	3.7	81.4	66.1	81.2	15.3
ϵ_M	0.12	19	2.8	1.8	1.5	0.2	2.5	2.1	0.2	0.5
C. Thyroidectomized, injected with anterior pituitary extract, exophthalmic										
19	5.6	545	92.7	70.1	15.4	7.2	104.8	87.2	83.1	17.6
ϵ_M	0.15	18	5.3	5.1	1.0	0.4	2.9	2.6	0.3	0.5
D. Thyroidectomized, injected with anterior pituitary extract, non exophthalmic										
9	3.6	538	60.2	40.0	14.5	5.7	92.5	75.6	81.7	16.9
ϵ_M	0.08	38	3.2	2.2	2.1	0.3	4.6	3.8	0.2	0.9
E. Thyroidectomized, injected with control extracts										
Kidney										
3	3.4	453	39.0	25.9	9.8	3.3	76.3	62.2	81.5	14.1
Heart										
4	3.5	407	37.7	27.9	6.2	3.5	68.5	56.2	82.0	12.4
Spleen										
3	3.5	417	42.4	31.2	7.3	3.9	81.6	66.5	81.4	15.1
Uninjected										
6	3.8	429	36.7	25.6	7.9	3.1	75.1	60.9	81.0	14.1

$$\epsilon_M = \sqrt{\frac{\Sigma d^2}{n(n-1)}}.$$

per cent in the weight of the orbital fat and of 17 per cent in the weight of the extraocular muscles of the animals receiving anterior pituitary extract, but which did not become exophthalmic (table 1).

[3] "P" represents the probability that the difference between the two means is due to random sampling.

The water content of the orbital fat increased from 24.8 mgm. in the normal control animals to 70.0 mgm. in the exophthalmic animals. This tissue normally contains a large amount of water, but in the exophthalmic animals water accounts for 75 per cent of the weight. The water content of the extraocular muscles of these animals was also increased (37 per cent, $P < 0.01$). Ablation of the thyroid gland even when no extracts were injected caused some increase of doubtful significance (4.6 mgm.) in the water content of the orbital fat but none in the muscle tissue. Injection of the control extracts had no effect on the amount of water in either the orbital fat or muscles. However, the water content of the orbital fat of the animals which received pituitary extract, but which failed to become exophthalmic, was slightly increased over that of the thyroidectomized control animals (36 per cent).

The data show clearly that the injection of the anterior pituitary or of the control extracts caused no increase in the lipid constituent of the orbital fatty tissue. In the control extract injected series it will be noted that the amount of lipoidal material is less than that from the animals in the untreated control groups (table 1 A, B and E.). This is presumably due to the use of smaller animals as recipients of the control extracts.

The amount of fat tissue residue after the removal of water and lipids was increased from 3.3 mgm. in the normal controls to 7.2 mgm. in the exophthalmic animals. This increase was consistent and was highly significant statistically. The weight of the dry residue of the ocular muscles was also increased (15 per cent, $P < 0.01$) in the exophthalmic animals, but the extent of the increase was much less than that of the water. There was no increase in the dry tissue residue of either the orbital fat or muscle as a result of thyroidectomy or the injection of control extracts. Since both water and solid components increase in amount in normal muscle hypertrophy, the proportion of water in the extraocular muscles of the exophthalmic and control animals was determined. The data (table 1) show that the eye muscles of exophthalmic guinea pigs contained a higher percentage of water than did those of the various control animals. The difference in water content per unit weight, although small, was very consistent and therefore has a high degree of significance ($P < 0.01$).

Non-orbital tissues. In contrast to the hypertrophy of the orbital fat caused by the injection of the pituitary extract, a decrease in the weight of the peritoneal fat was observed (table 2). Injection of the control extracts or thyroidectomy without the injection of extracts, however, did not change the amount of this fat nor the proportion of water in it relative to that of the normal controls.

The peritoneal fat of the pituitary extract-injected exophthalmic animals contained 55 per cent more water than did similar fat of the thyroidectomized controls (table 2 C). This increase, though definite ($P < 0.01$), was much less than the increase of 138 per cent found in the water content of the orbital fat. There was no change in the water content of typical skeletal muscle (table 2) of these animals. The mean values obtained suggest that thyroidectomy followed by the injection of the hypophyseal extracts tended to increase the

water content. However, statistical analysis reveals that none of the differences is significant. This is in direct contrast to the marked increase in water in the eye muscles. It is noteworthy that the normal eye muscles contained more water than did the skeletal muscles with which they were compared. Injection of the control extracts was without effect on the skeletal muscle water content,

TABLE 2

Comparison of water content of the non-orbital tissues of control and exophthalmic guinea pigs

NUMBER OF CASES	BODY	PERITONEAL DEPOT FAT		BODY MUSCLE WATER
		Total	Water	
A. Normal control group				
	grams	*grams*	*grams*	*per cent*
11	565	15.2	1.7	77.9
ϵ_M	34	2.4	0.2	0.4
B. Thyroidectomized, uninjected control group				
18	521	16.2	1.8	78.3
ϵ_M	21	1.8	0.1	0.3
C. Thyroidectomized, injected with anterior pituitary extract, exophthalmic				
16	517	10.9	2.8	79.2
ϵ_M	18	1.1	0.3	0.4
D. Thyroidectomized, injected with anterior pituitary extract, non exophthalmic				
9	538	11.7	2.1	77.8
ϵ_M	38	2.0	0.6	0.6
E. Thyroidectomized, injected with control extracts and uninjected controls				
Kidney 3	453	7.5	1.9	78.7
Heart 4	407	7.2	0.9	79.6
Spleen 3	417	6.4	1.0	80.1
Uninjected 6	429	6.9	1.3	80.2

as shown by comparison with the appropriate controls. The slightly higher values obtained in this group were due, it is believed, to the use of younger and smaller animals (table 2 E).

Discussion. The data obtained in this study show that the increased weight of the orbital fat of exophthalmic guinea pigs is due almost entirely to an excessive accumulation of water in this tissue. This increased weight of the orbital fat, therefore, is not the result of a true hypertrophy, since the main

cellular constituent, the fat cells, remains unchanged in amount as judged by the weight of the ether soluble lipids. Since there was no increase in lipids, it may be assumed that the framework of the fat cells did not increase in amount. Histological studies have shown an increase in the connective tissue in the orbital fat of exophthalmic animals; therefore it seems probable that the increase in weight of the "residue" fraction of the orbital fat tissue was almost entirely the result of connective tissue proliferation.

Since there was no increase in the total weight of the peritoneal fat of the exophthalmic animals, the high percentage of water in this tissue must have been obtained at the expense of either the lipid or connective tissue framework. These changes were without effect on body weight. The increase in water content of the peritoneal fat suggests that the edema of the orbital fat in experimental exophthalmos is an extreme example of a condition which is, however, not entirely restricted to the orbit.

The water content of the peritoneal fat was more variable than that of the orbital fat. This may have been due in part to the greater range in weight of the peritoneal fatty depots, since the smaller fat deposits contained proportionately more water than did the larger ones. This relationship is illustrated by the data on the water content of the peritoneal fat of the two uninjected thyroidectomized control groups (table 2 B and E). It is also evident that the water content of the orbital and peritoneal fatty tissue of normal guinea pigs differs greatly (peritoneal fat 11 per cent water and orbital fat 57 per cent water). A structural basis for this difference exists, for examination of sections of these tissues has shown that the orbital fat of normal and thyroidectomized guinea pigs possesses a large amount of loose connective tissue which contains an appreciable quantity of interstitial fluid. The fatty tissue which lies about the kidney and in the uterine mesentery, however, has very delicate septa, containing no observable interstitial material.

Hypertrophy of the extraocular muscles has been found to occur in both clinical and experimentally produced exophthalmos. Histological examination has shown that the eye muscles from such cases were extremely edematous (3, 6, 13). The present data demonstrate that nearly all of the increase in weight of the eye muscles of exophthalmic guinea pigs was due to an increase in the water component. However, there was a slight increase in the dry muscle residue, which suggests that some of the water increase was simply the result of muscle growth. This is in agreement with those experiments in which it has been demonstrated that the proptosis itself causes a hypertrophy of the eye muscles, presumably in response to the greater work required of them as retractors of the bulb (11). However, the percentage of water content was higher in the eye muscles of the exophthalmic guinea pigs, indicating that the increase in weight of these muscles was not entirely due to a simple growth hypertrophy.

Since no similar change in the percentage of water in the skeletal muscles was found, the edema of the eye muscles in exophthalmos appears to be a more localized reaction than the similar change in fat tissues. It is noteworthy

that the data on water content of skeletal muscles were based on pooled specimens of many muscles, some of which may have had an increased water content, as did the eye muscles, but which were insufficient in mass to affect the total value. There are at present no histological data available which suggests that the pathological state of the eye muscles in experimental exophthalmos represents a more general condition, excepting a reference by Paulson (8) to changes in the orbicularis oculi of exophthalmic guinea pigs. Examination of sections of diaphragmatic muscles of exophthalmic guinea pigs in our experiments has not revealed any abnormalities.

It is presumed that the cause of the increase in water content is the same in both fat and muscle. An increased vascular permeability may possibly be the factor immediately responsible for it. That contraction of Müller's orbital muscle or growth of the harderian gland does not cause the edema by interference with the vascular drainage of the orbit has been shown in earlier experiments (12). The finding that the water content of non-orbital tissues is also increased may be correlated with clinical observations of localized edema in non-orbital tissues of persons with exophthalmos (2, 4, 5).

CONCLUSIONS

1. Injection of an extract of anterior pituitary glands into thyroidectomized guinea pigs causes an exophthalmos and a hypertrophy of the orbital fat, which is almost entirely due to an increase in the water content of this tissue.
2. No change occurs in the lipid content of the orbital fat of these exophthalmic animals.
3. There is a marked increase of connective tissue in the orbital fat of exophthalmic guinea pigs.
4. The hypertrophy of the extraocular muscles which occurs in experimentally produced exophthalmos is largely caused by an increase in the water component. Some of the increase in water, however, is apparently due to normal growth.
5. The percentage of water found in pooled specimens of skeletal muscle of exophthalmic guinea pigs is not significantly greater than that in muscles from normal and thyroidectomized control guinea pigs.
6. In contrast to the orbital fat, the peritoneal fat depots do not increase in weight in exophthalmic animals, but the injection of anterior pituitary extract into thyroidectomized guinea pigs causes an increase in the water content of the peritoneal fatty tissues.
7. The orbital fat contained much more water than the peritoneal fat in normal and thyroidectomized guinea pigs.
8. Ablation of the thyroid gland had no effect on the weight of the extraocular muscles, orbital and peritoneal fat, or on the water content of the muscles and peritoneal fat, but did cause a slight increase in the water component of the orbital fat tissue.

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THE ABSENCE OF PHOSPHATE TRANSFER IN OXIDATIVE MUSCULAR CONTRACTION[1]

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When striated muscle contracts in the presence of an inadequate oxygen supply, the accumulation of lactic acid is accompanied by a loss of phosphocreatine (PC) and an accumulation of inorganic phosphate. At high rates of contraction there is also an accumulation of hexosemonophosphate (HMP). The amount of adenosine triphosphate (ATP) usually does not change. In mammalian muscle contracting with normal blood supply, these phosphate changes apparently stop when the oxygen supply becomes adequate (3, 11).

The Embden-Meyerhof formulation of phosphorylating glycolysis, based on studies of enzyme systems in cell-free extracts, postulates a large number of phosphate interchanges between these compounds and numerous others which have been found only in such extracts, as integral parts of the formation of lactic acid. An alternative formulation has been presented (9) in which only the phosphate changes actually observed are considered to take place. According to this, the hydrolysis of the potassium salts of PC furnishes alkali to buffer the lactic acid, and the formation of HMP is interpreted as a secondary mechanism for supplying energy under anaerobic conditions when the glycolytic mechanism is insufficient.

Still a third formulation has recently been proposed by Needham et al. (6). This is based on the finding by Engelhardt and Ljubimova (2) that myosin acts as an enzyme in hydrolyzing ATP to adenosine *di*phosphate and inorganic phosphate. Needham and his co-workers have attempted to link the chemical and physical processes in contraction, by postulating the formation of myosin phosphate in this reaction, with the myosin in the extended state. In the contraction processes, the nerve impulse is considered to cause the liberation of inorganic phosphate from the myosin compound, with return of the myosin to the contracted state, and corresponding contraction of the muscle fibril. The ATP is then restored by some phosphate donor. Several possible donors are mentioned, but since PC is the only one present in quantity, this must be the ultimate source of phosphate for this resynthesis. Some other unspecified reaction process must then resynthesize the PC from inorganic phosphate.

Obviously, the over-all changes in the contracting muscle do not give any information on possible intermediate steps. On the other hand, before either the Embden-Meyerhof or Needham formulation can be accepted as valid, it is desirable that direct evidence for such interchanges of phosphate groups be found in the intact muscle. The use of radioactive phosphorus (P^{32}) as a tracer offers

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the means of determining whether such interchanges do take place, if conditions can be found in which the resting metabolism of muscle results in a differential distribution of P^{32} among the various organic compounds. If the Embden-Meyerhof cycle is operating in contraction, then stimulation should result in equalization of the distribution of P^{32} among the three organic compounds; if the mechanism postulated by Needham is active, an equalization should take place between the PC and ATP. On the other hand, if neither set of phosphate interchanges is involved in the contraction process, stimulation should not effect any such equalization in the distribution of the P^{32}.

In previous experiments using the tracer technique (8) it was found that a prolonged tetanic contraction under essentially anaerobic conditions did not affect the distribution of the P^{32}. However, evidence was obtained (10) that the course of resting muscle metabolism does involve interchanges of phosphate groups between PC, the two labile groups of ATP, and inorganic phosphate. It was therefore concluded that the phosphorylating glycolysis should be referred to the resting metabolism of muscle rather than to the metabolism of anaerobic contraction.

These findings do not exclude the possibility of such phosphate interchanges during oxidative contraction, without any over-all changes in phosphate distribution. To test this possibility, the effect of a prolonged series of single twitches on P^{32} distribution was determined. The experiments were performed on cats under pentobarbital anesthesia. The solution containing the P^{32} in the form of Na_2HPO_4 was injected subcutaneously, and the stimulation performed two hours later. Previous work (10) had shown that this time is optimum for obtaining a differential distribution of the P^{32}. One gastrocnemius muscle was subjected to 120 to 180 isometric twitches, by condenser discharges through the nerve, at the rate of 1, 2, or 3 per second for 1 or 2 minutes. This muscle and the companion resting muscle were then frozen, and the phosphate compounds isolated from trichloroacetic acid filtrates by the methods used previously (10). Measurements of relative radioactivity were made by a Geiger-Müller counter. The effect of the stimulation on the contents of inorganic phosphate, PC, ATP and HMP was also determined. In the table below these data are given, with the figures for P content of the stimulated muscles corrected for the uptake of water from the blood stream during the stimulation period. This was done by multiplying the observed values in milligrams per cent, by the ratio of total acid-soluble P of the resting and stimulated muscles.

The results show no evidence for phosphate transfers resulting from the stimulation. In every case in which the resting muscle shows an unequal distribution of P^{32} between any two of the organic compounds, this unequal distribution is retained in the companion stimulated muscle. The only changes in P^{32} distribution are those indicated by the changes in distribution of the total P. Thus neither in anaerobic contraction nor in contraction under oxidative conditions is there any evidence for either the Embden-Meyerhof or the Needham cycle of phosphate interchanges. These data add considerable weight to the conclusion previously indicated (10), that there is a qualitative difference in the

pathways of carbohydrate utilization in resting metabolism and in contraction. Other data in support of this view are the finding (7) that the point at which iodoacetic acid inhibits lactic acid formation in anaerobic contraction is different from that at which the reactions in extracts are inhibited, and the important

TABLE 1

Effect of repeated isometric twitches on distribution of P^{32} and of total P in muscles of cats

In each space the upper figure represents radioactivity, as counts per minute per milligram of P. Lower italicized figure gives P content in milligrams per cent. R = Resting muscle. S = Stimulated muscle.

CAT NO.	INORGANIC P	PHOSPHOCREATINE	ADENOSINE TRIPHOSPHATE (2 LABILE GROUPS)	HEXOSEMONOPHOSPHATE	STIMULATION	
					Rate per second	Duration minutes
1 R	565 *24*	99 *70*	156 *37*	260 *9*		
1 S	352 *35*	108 *37*	181 *23*	243 *9*	1	2
2 R	530 *23*	51 *49*	161 *42*	83 *12*		
2 S	385 *47*	82 *26*	157 *44*	72 *14*	1	2
3 R	392 *17*	42 *61*	145 *26*	89 *10*		
3 S	262 *36*	34 *50*	87 *27*	47 *10*	1	2
4 R	258 *27*	23 *61*	26 *38*	147 *10*		
4 S	130 *41*	34 *39*	33 *28*	95 *15*	2	1
5 R	505 *18*	84 *58*	73 *38*	107 *10*		
5 S	321 *49*	103 *18*	64 *38*	100 *30*	3	1

observation by Stannard (12) that azide inhibits completely the excess oxygen uptake by frog muscle resulting from contraction, in concentrations which do not affect the oxygen uptake of resting muscle.

In the previous experiments (8) no account was taken of the possible difference in P^{32} content of extracellular and intracellular inorganic phosphate. Attention has been called to this by two reviewers (4, 5). However, the essential conclu-

sion indicated in that paper is not invalidated, for there too it was found that contraction did not modify the P^{32} distribution between any two of the organic compounds. The study of resting muscle with this tracer (10) has shown that inorganic phosphate enters into the intracellular inorganic phosphate only by hydrolysis of PC or ATP formed at the membrane. Hence the level of P^{32} in the intracellular inorganic P does not exceed that in PC or ATP. The interpretation there given has been strengthened by the results of Bollman and Flock (1). They found that in rats treated with P^{32} the resynthesis of PC in recovery from contraction did not raise the P^{32} level of the PC beyond that of the resting muscle. The very high P^{32} content of the muscle inorganic phosphate is therefore ascribable to the extracellular phase and the unavoidable inclusion of some blood in the muscle sample.

In agreement with the finding of Bollman and Flock, the present data show no evidence for an increased uptake of P^{32} during the stimulation period. This point again argues against the view that the phosphate interchanges are a fundamental part of the metabolism of contracting muscle.

The point of view here presented does not in any way invalidate the experimental finding of Engelhardt and Ljubimowa that myosin is the enzyme which hydrolyzes ATP. Since there is obviously a breakdown and resynthesis of ATP in resting metabolism, the experimental data show that such an enzyme is operating in resting metabolism. However, this observation, which seemed at first to offer a link connecting the chemical and physical processes in contraction, fails to meet the test of experiment.

The radioactive phosphorus used in these experiments was supplied by the Department of Physics of the University of Michigan.

SUMMARY AND CONCLUSIONS

1. The effect of a prolonged series of twitches on interchanges between the phosphate compounds of muscles has been studied by means of radioactive P.

2. Such contractions, under essentially oxidative conditions, do not bring about interchanges between phosphocreatine, adenosine triphosphate, or hexosemonophosphate.

3. Under these conditions, the contraction process does not result in any increased exchange of intracellular phosphate groups with plasma inorganic phosphate.

4. The evidence obtained indicates that neither the Embden-Meyerhof phosphorylating glycolysis nor the enzyme function of myosin are concerned directly with the chemistry of muscular contraction.

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THE PERSISTENCE OF HEAT ACCLIMATIZATION IN MAN[1]

AUSTIN HENSCHEL, HENRY LONGSTREET TAYLOR AND ANCEL KEYS

From the Laboratory of Physiological Hygiene, University of Minnesota, Minneapolis

Received for publication August 16, 1943

When man is subjected to high environmental temperature a series of physiological adjustments occurs which reduces the debilitating effects of the heat. The major adjustments take place during the first few days in the heat (1). Heat acclimatization is obviously lost in a cool environment but the rate at which this happens seems to be little known. A survey of the available literature has failed to reveal any reports specifically concerned with this problem. The persistence of heat acclimatization is of importance in many practical situations, notably in military operations.

This paper is a report on 4 series of experiments involving 24 men who were studied at high temperatures on two occasions from 1 to 4 weeks apart. The environment, physical work, and diet were rigidly controlled.

Subjects. The subjects were normal young college students free from physical defects that might influence cardiovascular adjustments. Ages ranged from 19 to 28 years.

The subjects remained continuously in the air-conditioned suite at the Laboratory from the afternoon preceding each exposure to high temperatures to the end of each experiment. During the 1 to 4 week intervals between exposures to the heat the subjects were not at the Laboratory. The experiments were performed during the winter months so that the subjects encountered high temperatures only during the experiments.

PROCEDURE. A standard regimen of observations, physical work, rest and diet was maintained in the 4 series. The length of the intervals between exposures to the heat was 1 week in series I, 2 weeks in series II, three weeks in series III and 4 weeks in series IV.

For each experiment the routine consisted of work during two successive afternoons at 78°F. followed by two full days of work at temperatures of 110°F. in the mornings and 120°F. in the afternoons. Night temperature was 85° to 90°F. Relative humidity was held constant at 20 to 25 per cent saturation.

The physical work consisted of walking on a motor-driven treadmill at a constant rate of 3.25 miles per hour and a 7.5 per cent angle of climb. Work of this intensity demands an oxygen consumption at about 7 times the basal rate. Each half day's work consisted of 6 ten-minute work periods alternating with

[1] This work was supported in part under the terms of a contract, recommended by the Committee on Medical Research, between the Regents of the University of Minnesota and the Office of Scientific Research and Development. Important financial assistance was also provided by the Nutrition Foundation, Inc., by the U. S. Cane Sugar Refiners' Association, N. Y., by the Corn Industries Research Foundation, N. Y., by the National Confectioners' Association, Chicago, and by Swift and Co., Chicago.

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ten-minute rest periods. Standard clothing of cotton shorts, shoes and socks was used for work and one-piece U. S. Army Armored Force battle dress (cotton drill) was used during the evenings.

Pulse rates were counted before and during the first 15 seconds immediately after each work period. Rectal temperatures were taken with a clinical thermometer for the first 90 seconds after the third and sixth work period each morning and afternoon. Cramptom (2) "blood ptosis" tests were made before and after each days' work. Body weights were recorded each morning before breakfast and with an empty bladder. The subjects were weighed to $\pm\frac{1}{4}$ ounce nude and dried before and after the third work period each morning and the sixth

TABLE 1

The grand averages of the physiological variables measured in the four series of experiments for the 1st and 2nd exposures to the heat

ROOM TEMP.	WORK PULSE RATE-BEATS/MIN.					RECTAL TEMPERATURE, °F.					CRAMPTON SCORE*				
	78° F.	110° F.	120° F.	110° F.	120° F.	78° F.	110° F.	120° F.	110° F.	120° F.	78° F.	90° F.	120° F.	90° F.	120° F.
	p.m.	a.m.	p.m.	a.m.	p.m.	p.m.	a.m.	p.m.	a.m.	p.m.	p.m.	a.m.	p.m.	a.m.	p.m.
Series I															
1st exposure	112	134	145	126	136	100.3	99.9	100.5	99.6	100.4	71	71	56	64	59
2nd exposure	109	127	132	127	126	100.2	99.7	100.1	99.5	99.9	67	58	65	70	63
Series II															
1st exposure	109	132	139	122	140	100.1	99.7	100.4	99.8	100.4	68	66	44	54	62
2nd exposure	116	123	123	117	125	99.9	99.6	99.9	99.6	100.1	66	77	52	69	66
Series III															
1st exposure	117	141	151	135	155	100.1	100.6	101.1	100.5	101.5	75	76	46	64	50
2nd exposure	112	140	149	134	142	100.2	100.3	101.0	100.3	100.8	69	71	49	64	64
Series IV															
1st exposure	116	138	140	129	137	99.9	100.3	100.5	100.2	100.4	79	64	44	60	64
2nd exposure	113	136	139	128	142	100.1	99.8	100.3	99.6	100.4	63	65	46	54	61

* A higher score means a better adjustment.

work period each afternoon for rates of sweating. Twenty-four hour urine output and fluid intake volumes were recorded for the two days in the heat.

A constant diet was provided by a trained dietitian for three days preceding and during each experiment. The diet contained about 3,100 calories and was normally balanced in carbohydrate, protein and fat. It was adequate according to current standards in all the vitamins and provided 15 ± 2 grams of sodium chloride daily. Water was allowed *ad lib.*

RESULTS. The grand averages for the physiological variables measured and the statistical evaluation of the mean (t-values, Goulden, 3) are given in tables 1 and 2. Each half day's pulse rate represents the average of from 24 to 48 individual work pulse rate counts. Similarly the rectal temperature values for each half day represent the average of 12 to 24 determinations.

The work pulse rates were significantly lower on the second exposure for both the first and second afternoons in the heat in series I and II. In series III the

advantage of having been partially heat acclimatized three weeks previously showed up only on the second afternoon in the heat. For the four week interval (series IV) there was no hold-over effect. The morning work pulse rates in the heat and the afternoon values at normal temperatures (78°F.) were not significantly different between the first and second exposures in any of the series.

The rectal temperatures exhibit the same progressive decline in hold-over effect as do the work pulse rates. The rectal temperature differences are significant for both the first and second afternoons in the heat when the interval between exposures is only one week (series I). The two and three week interval groups (series II and III) are improved on either the first or second afternoon in the heat but the four week group (series IV) was not improved on the second exposure.

In general the Crampton test demonstrated no benefit derived from a previous exposure to heat except on the first afternoon in series I. Even though the t-values are not significant there is, however, a progressive trend toward a lower t-value as the interval between the two exposures to heat is greater. The Crampton test has previously been found to be a less sensitive index of the state of acclimatization than are the work pulse rates and rectal temperatures (1, 4).

TABLE 2

t-values for the differences between the means of the physiological variables for the two exposures to the heat on the 1st and 2nd afternoons

A negative t-value indicates a higher mean obtained on the second exposure to the heat

	WORK PULSE BEATS/MIN.		RECTAL TEMP., °F.		CRAMPTON SCORE		t-VALUES FOR	
	1st p.m.	2nd p.m.	1st p.m.	2nd p.m.	1st p.m.	2nd p.m.	1% level	5% level
Series I	5.613	7.752	3.279	3.333	−2.388	−1.01	3.50	2.36
Series II	3.543	6.122	4.10	1.957	−0.959	−0.364	4.60	2.78
Series III	2.05	5.312	0.653	5.035	−0.421	−1.879	3.71	2.45
Series IV	0	1.935	0.649		−0.452	0.322	5.84	3.18

The grand averages for the observations on water balance during work in the heat are given in table 3. There was no significant difference or trend in the rate of sweating during work, fluid intake, urine output or weight loss between the first and second exposure to the heat in any of the 4 series. The absolute values are about the same as those observed by the authors in other studies when the work output and temperatures were comparable. It is, however, interesting that in series I and II the calculated total sweat volume was nearly 1½ liters less during the second exposure to the heat. In series III it was only slightly decreased while in series IV it was ½ liter greater.

Discussion. We have noted the absence of previous reports on the persistence of heat acclimatization. In the popular press there have been statements that German troops destined for hot weather service in the African deserts were "conditioned" in heated buildings in Germany. From the present results it is

clear that such preparation might be of real value even though as long as several weeks elapsed between acclimatization in Germany and the operation in Africa.

TABLE 3

Average values for water balance during the two days in the heat

	RATE OF SWEATING, GRAMS/MIN.				WATER INTAKE	URINE OUTPUT	BODY WEIGHT CHANGE	CALCULATED TOTAL SWEAT*
	110°F., a.m.	120°F., p.m.	110°F., a.m.	120°F., p.m.				
					liters	*liters*	*ounces*	*liters*
Series I								
1st exposure	9.3	12.6	8.1	12.6	9.713	1.669	+6	8.700
2nd exposure	9.5	11.9	10.8	13.6	9.841	2.300	+32½	7.390
Series II								
1st exposure	9.6	11.6	10.2	12.2	10.110	2.477	+22	7.722
2nd exposure	8.2	13.1	9.1	12.3	6.845	1.630	−4½	6.343
Series III								
1st exposure	11.7	13.2	13.2	14.6	9.937	1.707	−12½	9.382
2nd exposure	12.1	13.3	13.3	13.7	11.009	2.829	+7½	8.839
Series IV								
1st exposure	9.8	12.5	11.2	13.3	9.539	2.350	+10½	7.640
2nd exposure	9.7	11.2	10.1	12.2	9.842	2.156	+17	7.952

* Total sweat = (water drunk + water in food + water of metabolism) − (urine output + water in feces + water in expired air) ± (body weight change during the two days in the heat).

TABLE 4

The number of individuals showing a change in physiological variables between the two exposures to the heat on the 1st and 2nd afternoons of work

	WORK PULSE RATE CHANGE GREATER THAN 5 BEATS/MINUTE			RECTAL TEMPERATURE CHANGE GREATER THAN 0.2°F.			CRAMPTON SCORE CHANGE GREATER THAN 5		
	Increase	No change	Decrease	Increase	No change	Decrease	Increase	No change	Decrease
Series I									
1st p.m. heat	0	1	7	0	2	6	4	3	1
2nd p.m. heat	0	1	7	0	1	7	5	3	0
Series II									
1st p.m. heat	0	0	5	0	0	5	2	2	1
2nd p.m. heat	0	0	5	0	2	3	2	2	1
Series III									
1st p.m. heat	0	7	0	2	1	4	4	2	1
2nd p.m. heat	0	0	7	0	0	7	6	1	0
Series IV									
1st p.m. heat	1	2	1	1	0	3	2	1	1
2nd p.m. heat	3	1	0	2	0	2	1	1	2

We have no information as to the persistence of acclimatization after a first exposure of less than or more than the 2-day period used here but it may be presumed that a longer exposure might result in somewhat more persistent ac-

climatization. Another question of interest would be the possible accumulation of ability to acclimatize to the heat through repeated exposures.

Individuals differ markedly in their response to work in heat but individual variations were not prominent in these studies on persistence of heat acclimatization. Table 4 gives the number of individuals in each series who showed a change in the physiological variables between the first and second exposures to heat. "Real" differences were arbitrarily considered to be changes of 5 beats per minute for work pulse rate, 0.2°F. for rectal temperature and 5 points for the Crampton score. Work pulse rate is the most sensitive index of the condition of the subject in the heat. The uniformity of behavior of this variable in the several subjects is notable in the first 3 series. The rectal temperature is less reliable and the Crampton test is the least consistent variable studied.

The detailed nature of the changes in acclimiatization to heat is unknown but they are exhibited largely in cardiovascular effects and the principal components are the heart, the blood volume, and the constriction and dilatation of the appropriate blood vessels. Presumably these are of increasing importance in the order named. Accordingly it would seem that the autonomic nervous system dominates the picture.

SUMMARY

1. The performance of muscular work in dry heat was studied in 24 normal young men. The diet, environment and work routine were carefully controlled during the stay in the heat.

2. Comparisons were made between the performances in the heat on two occasions of 2 days each separated by from 1 to 4 weeks of cold weather.

3. Work pulse rates, rectal temperatures and, to a lesser degree, vasomotor stability tests showed that heat acclimatization persists during at least three weeks of cold weather. The benefit conferred by this acclimatization is important for this time but the advantage decreases from week to week.

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SOME EFFECTS OF PECTIN SOLUTIONS DURING POSTHEMORRHAGIC HYPOTENSION[1]

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The use of an autoclaved pectin solution as a blood substitute has been advocated by Hartman and associates (1) on the basis of its colloidal osmotic pressure and its capacity to restore effective circulating volume and arterial pressure after hemorrhage and experimental procedures causing shock.

This report is concerned (a) with the question whether such solutions administered after standard periods and degrees of posthemorrhagic hypotension prevent the development of an irreversible state, and (b) with an analysis of hemic and hemodynamic actions following their use under such conditions.

Preparation. A 1.5 per cent solution of autoclaved pectin kindly supplied by Frederick Stearns & Co., Detroit, was used in these experiments. Various shipments were stated to have a viscosity of 3.58 to 4.86, and osmotic pressures between 67 and 68.7 cm. H_2O. The micellar aggregate size was about 60,000. In some experiments solutions with a pH of 3.68 to 3.9 were brought to 6.9 to 7.2 by phosphate buffers recommended by Hartman et al.; in other experiments a buffer supplied with the pectin and containing glycine, lactate and sodium hydroxide was added in recommended amounts. These brought solutions to a pH of 6.6.

PART I. *The substitute value of pectin solutions.* Since conclusions as to the substitute value of a solution depend not only on its properties but also on the conditions under which it is tested, the procedure used and the reasons for its adoption must be briefly stated.

Dogs anesthetized with morphine and biologically standardized doses of sodium barbital (2) were bled at a rate of 50 ml./min. until mean arterial pressure was reduced to 50 mm. Hg. Any tendency to increase was neutralized by bleeding a little more from time to time. In this way, a 50 mm. hypotension period was maintained in different animals from 30 to 120 minutes. If no infusion was given after 30 minutes, arterial pressures failed to recover. If, however, the animal's own blood (heparinized) was infused after 90 minutes of such hypertension, 90 per cent of animals recovered. Indeed, the small percentage of deaths can probably be accounted for by failure fully to prevent certain hazards in the drawing and keeping of such blood (inadequate heparinization, faulty filtration, etc.). Not only was aortic pressure restored but the pressure pulses maintained a normal form for 6 to 8 hours after such infusions. With such

[1] This investigation was supported by a grant from the Commonwealth Fund.

[2] Rockefeller Travelling Fellow.

actions of a presumably ideal transfusate after large hemorrhages, the effects of autoclaved pectin solutions were compared.

The procedure was chosen 1, because previous experiments (3) indicated that any damaging influence of severe hemorrhage is correlated better with the degree and duration of the hypotension induced than with the quantity of blood withdrawn, even when bleeding occurs at constant rates, and 2, because it afforded variable periods for development of the damaging effects of a standard hypotension. Furthermore, since a good blood substitute should not merely restore and maintain the effective circulating volume and arterial pressure, but should also prevent the development of an irreversible state under conditions in which the animal's own blood does so, this method seems to offer a test which is more nearly comparable with situations arising in practice than procedures in which blood is immediately replaced after a hemorrhage. It seems highly improbable that, in practice, a large hemorrhage can be checked and a transfusion given in less than the minimal interval of 30 minutes used in these experiments.

Results. In various experiments pectin infusions were given at a rate of 3–5 ml./min. after existence for 30, 50, 90 or 120 minutes of a 50 mm. post-hemorrhagic hypotension. Even faster rates of infusion were tolerated by normal animals without adverse effects, as revealed by optical pressure curves and electrocardiograms. Nor were gross pathologic disturbances seen in any organ at autopsy.

Thirty-two dogs, exclusive of controls, were used. In 19 of these, the volume of pectin solution practically equaled the volume of blood withdrawn; in 7 it was about 65 per cent of the blood lost. In 6 dogs, 80 per cent of the blood was replaced by pectin and this *was immediately followed* by an infusion of 20 per cent of the animal's own heparinized[3] blood.

Of the 32 dogs, 10 recovered from pectin infusions, 17 died from a precipitate type of circulatory failure and 5 after a delayed failure. A "breakdown" of these results is of interest. In 1 experiment the infusion was started after two hours of 50 mm. hypotension; in 3, after 90 minutes and in 7, after 40 to 49 minutes. All of these animals died quickly. In 3 animals which developed a precipitate shock following pectin infusions, the animal's blood was reinfused. This only revived the animals temporarily. This is additional evidence that an irreversible state had developed despite infusion of pectin solutions. On the other hand, of the 19 animals in which infusion began after 30 minutes of hypotension, a satisfactory dynamic recovery of the circulation occurred in 11 while a precipitate or delayed circulatory failure occurred in 8 dogs. It seems apparent that the usefulness of autoclaved pectin solutions after severe hemorrhage is restricted to the early periods of hypotension which follow.

For this reason, only the latter experiments merit further analysis. Details of these are given in table 1 and plots of representative experiments illustrating circulatory recovery, delayed and precipitate circulatory failure are shown in figures 1, 2 and 3. In 6 experiments in which smaller blood transfusions followed

[3] We are indebted to Roche-Organon, Inc., Rahway, N. J. for the Liquaemin used in these experiments.

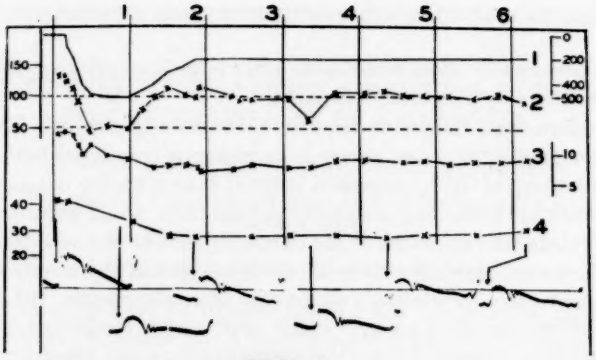

FIG. 1

FIG. 2

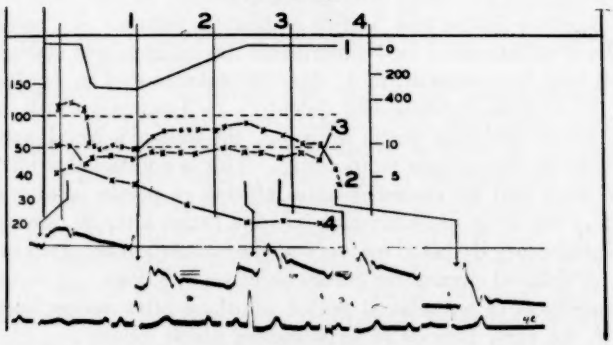

FIG. 3

Fig. 1. Circulatory recovery.

Fig. 2. Delayed circulatory failure.

Fig. 3. Precipitate circulatory failure. Plots in all indicate 1, amount and rate of blood withdrawn (downstroke) and volume and rate of pectin infused in milliliters; 2, mean arterial pressure in millimeters of mercury; 3, effective venous pressure in centimeters saline; 4, hematocrit readings as percentage of red cells. Below segments of central pressure pulses taken at times indicated by arrows and in figure 3 an e.c.g.

use of pectin certainly no incompatibilities developed; on the contrary, these infusions appeared to be beneficial.

PART II. *Hemic effects of pectin solutions.* Small samples of arterial blood were drawn periodically to study blood changes. Rapid sedimentation rates and pseudoagglutination (microscopic and macroscopic evidence of clumping) were found in blood mixed with pectin in the body as well as in vitro. However, no postmortem evidence of stasis, congestion, infarction, etc., was found in survival animals. Animals that went into shock usually revealed duodenal mucosal changes which were similar to but paler than those of animals dying without such infusions (3).

As shown in the plots of figures 1, 2 and 3, the arterial hematocrit readings were reduced after a 30 minute period of hypotension, as anticipated. In a few cases (expts. 2, 13, 14, 15, fig. 1, columns 1, 2) the decrease may not have been significant and in two instances (expts. 1, 18) an apparent concentration occurred. These few exceptions bring up the question whether stock animals used for such experiments are always in an adequately hydrated state. In three animals (expts. 6, 7, 8), the spleen was exteriorized and while emptied of its blood by a small dose of epinephrine, its pedicle was clamped. In all of these animals, hemodilution occurred after hemorrhage, indicating that this organ is not responsible for changes observed after hemorrhage. These observations are sufficient to indicate that at the time pectin solutions were injected, a state had not been reached when fluid was leaving the circulating system; on the contrary, the flow of water seemed to be toward the blood stream.

The effects on hematocrit readings 3 to 5 minutes after recirculation of the infused pectin and at the end of 4 to 6 hours are shown in figures 1, 2 and 3 and in columns 3, 4 of table 1. The first serves as a rough index of the effective dilution due to the injected solution itself; the latter gives some idea as to osmotic capacity for attracting water exerted by colloidal pectin a few hours after infusion. In each of the experiments in which pectin only was injected (expts. 1–13) a tremendous additional dilution occurred at once. Red cell volumes decreased 13.9 to 19.3 per cent in all except the three splenectomized animals in which they decreased only 6, 8.6 and 8.4 per cent respectively. Curiously, an equal or even greater reduction occurred in the five experiments in which an infusion of some blood followed use of pectin solutions (expts. 15–19).

Many plots similar to those of figure 1, 2 and 3 showed, with few exceptions, that no further reduction in hematocrit values occurred, and at the end of 4 to 6 hours they increased gradually. Of course, the readings were much less than at the beginning (cf. columns 1 and 4, fig. 1). The significance of such observations must be tempered by the knowledge that animals under morphine and barbital ordinarily show a progressive hemoconcentration of even greater magnitude (4). However, if injected pectin solutions exert any osmotic attraction for tissue fluids after hemorrhage this must be small. These reactions following severe hemorrhage are apparently different from those observed on normal animals and man (1, 5). On the other hand, the degree of dilution achieved by its infusion seems to be maintained for 4 to 6 hours.

A review of our results shows, however, that the persistence of low hematocrit readings does not correlate with recovery of blood pressure in our experiments; on the contrary, animals which showed the greater tendency to return to normal concentrations were the ones that recovered (cf. expts. 1, 2, 4, 5, 13, 18, with expts. 7, 8, 9, 10, fig. 1). While no relation of increasing hemoconcentration to recovery can be claimed, the results strongly suggest that the ability of a solution to maintain hemodilution cannot be used as a sole criterion of its dynamic usefulness.

TABLE 1

EXP.	HEMORRHAGE	DURATION 50 MM. HYPOTENSION	PECTIN INFUSION	BUFFER	HEMATOCRIT				OUTCOME
					1	2	3	4	
	ml./kilo		*ml./kilo*						
1	30	35	30	Phosphate	39.0?	45.7	26.4	33.4	Recovery
2	38	31	38	Phosphate	46.7	42.3	28.4	33.7	Recovery
3	47	35	47	Phosphate	54	51.7	51.7	31.3	Recovery
4	46	29	46	Phosphate	49.0	43.0	27	37	Recovery
5	42	31	40	Phosphate	51	48	22	35	Recovery
6*	38	30	23	Phosphate	42.7	33.5	27.5	30.0	Recovery
7*	28	32	28	Phosphate	45.2	34.1	25.5	21.2	Precip. shock
8*	5.5	30	11	Phosphate	40.5	38.3	29.9	29.7	Precip. shock
9	32	34	33	Phosphate	42.4	36.8	23.1	20.9	Precip. shock
10	40	32	25	Phosphate	49.3	42.3	29.5	28.2	Precip. shock
11	36	31	32	Phosphate	58.5	52	34.5	38.5	Precip. shock
12	33	29	29	Phosphate	53	39	25	36	Precip. shock
13	33	31	33	Lactate	41.5	40	27	42	Recovery
14	40	30	20 + 20B	Lactate	47	40	31	36	Recovery
15	52	37	33 + 4B	Lactate	47.5	46.5	19	34	Recovery
16	33.5	33	40 + 10B	Lactate	50	46	22	34	Recovery
17	53	31	26 + 6B	Lactate	46.5	35	25	26.5	Delayed shock
18	39	25	32 + 6.4B	Phosphate	37?	41	26	33	Recovery
19	41	30	31 + 8B	Phosphate	41	35	24	34.5	Delayed shock

* Splenectomized dogs. Hematocrit readings—(1) control, (2) at end of 50 mm. period hypotension, (3) after infusion and recirculation of pectin, (4) 4–6 hours later.

PART III. *Electrocardiograms and hemodynamic reactions.* Electrocardiograms (standard leads) were recorded during the course of most experiments. Segments of one of these are incorporated in figure 3. No changes of significance occurred either in animals that recovered or those that failed. During the period of hypotension all deflections decreased in amplitude and during terminal slowing of the heart the P-R interval sometimes increased (fig. 3). In particular, no electrocardiographic evidence was obtained which suggested thrombosis or impairment of the coronary or pulmonary circulations. Optical records of central arterial pressure pulses were recorded in 8 animals.

Segments of such pressure pulses obtained in animals responding differently to pectin infusions are shown in figures 1, 2 and 3. In figure 1, from a dog which made a satisfactory hemodynamic recovery, an infusion of pectin solution

equal to 65 per cent of the withdrawn blood was given. Mean arterial pressure was not fully but satisfactorily restored and it was maintained during the period of observation. The pulse pressure following infusion was somewhat less than during the control period, but the form of the pressure curve was normal and remained so for 4 hours. Incidentally, this experiment illustrates during the third and fourth hour a frequent occurrence in such revived animals, viz., a temporary decline of arterial pressure, 60 to 90 minutes after completion of infusion. This was always followed by a secondary rise which was maintained. During such temporary drops the form of the pressure pulses did not deteriorate, and electrocardiograms showed no significant changes.

Figure 2 illustrates a type of experiment in which infusion of pectin solution was followed by a delayed circulatory failure. Immediately following injection the form of the pressure pulses was restored to normal. Gradually, this deteriorated, the curves being characterized by a large preliminary fling, a peaked systolic summit, deep incisura and progressively flattening diastolic part of the curve. These changes resemble those previously reported as characteristic of circulatory failure (3). It may be noted that in this experiment, as in others previously reported, circulatory failure developed despite gradually increasing effective venous pressure.

Figure 3 illustrates one of the experiments in which reinfusion of full amounts of pectin solution failed to restore the form of pressure pulses even temporarily and a subsequent rapid deterioration. It will be noted that in this experiment the rate of infusion was slower, but similar precipitate shock also occurred in experiments in which a more rapid rate was used.

Discussion. The data presented strongly suggest that infusions of pectin solutions are ineffective after a 30 minute period of a 50 mm. Hg post-hemorrhagic hypotension. Given at the end of 30 minutes, 11 out of 19 dogs showed satisfactory hemodynamic reactions. This series is too small to venture opinions on a statistical basis, but certain reactions, particularly in animals that did not respond favorably, suggest caution in the use of such solutions after severe hemorrhage. It should be emphasized that our deductions do not necessarily apply to states of hypotension produced otherwise than by loss of blood and particularly when these are accompanied by hemoconcentration.

We have confirmed many observations of previous workers, considered adequate for their clinical trial. Thus, after a post-hemorrhagic hypotension of not too long duration, their infusion causes an immediate increase in pulse pressure, restoration of arterial pressure and generally normal forms of pressure pulses. Hematocrit readings suggest that hemodilution occurs by virtue of the fluid injected and that a good degree of retention occurs for at least 4 to 6 hours. Evidence that blood volume is further increased by virtue of an osmotic attraction of water from tissues is lacking. However, this may be a virtue rather than a fault, for an excessive depletion of interstitial and cellular water may be harmful rather than beneficial after hemorrhage. Recent evidence (6) indeed suggests that cellular water is already utilized in the hemodilution which follows hemorrhage. However, no correlation existed between maintenance of an initial

dilution of blood by pectin and hemodynamic recovery which suggests that it is hazardous to rely on the demonstrated ability of pectin solutions—or for that matter any colloid—to remain within the blood stream as a satisfactory criterion of its efficacy. Hemodynamic recovery depends on more than the capacity of an infusate to exert an adequate oncotic pressure.

While no direct evidence was obtained either experimentally or at the necropsies that the agglutination and rapid sedimentation is harmful, the occurrence of a rapid decline in arterial pressure and deterioration of pressure pulses in some of the experiments and the failure to overcome this by subsequent large infusions of the animal's own blood certainly does not suggest that infusion of pectin solution delays the development of an irreversible circulatory state. It is conceivable, as Ivy et al. (7) suggest, that a tendency to intravascular clumping may reduce oxygen supply more seriously after severe hemorrhage than in animals having their normal quota of red corpuscles. Further experimentation, however, is required to establish this or other reasons for the too common unfavorable responses.

SUMMARY

1. A 1.5 per cent buffered pectin solution exerting an oncotic pressure of 67 to 68.7 cm. of water was injected at a rate of 3–5 ml. per minute after varying periods of post-hemorrhagic hypotension (50 mm. Hg).

2. The usefulness of such solutions is apparently limited to the early periods of such hypotension. Given after 30 minutes, 11 out of 19 dogs showed a satisfactory hemodynamic response for 4 to 6 hours after administration.

3. Pectin infusions, given after a post-hemorrhagic dilution of blood has already occurred, cause a further dilution by virtue of the fluid injected and this is rather well maintained for 4 to 6 hours. Evidence that additional dilution results from osmotic attraction of water from tissues was not found.

4. No correlation existed between maintenance of hemodilution and favorable hemodynamic reactions; on the contrary, the animals that recovered usually showed some tendency to reconcentration. This suggests that the demonstrated ability of a colloid *a*, to maintain an effective oncotic pressure over a considerable time interval, and *b*, to increase blood volume in normal animals is not a satisfactory criterion of its physiological usefulness.

5. While no evidence was obtained experimentally or at necropsy that the rapid sedimentation and agglutination produced by such solutions is harmful, the occurrence of a precipitate failure of the circulation in too many experiments and the inability to overcome this by subsequent large infusions of blood suggest that pectin infusions may exert some deleterious influence when used after severe hemorrhage. Consequently, caution should be exercised in the employment of pectin solutions in such conditions.

6. These conclusions do not necessarily apply to states of hypotension produced otherwise than by loss of blood and particularly when hemoconcentration exists.

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THE RENAL EXCRETION OF CHLORIDE BY THE NORMAL AND BY THE DIABETES INSIPIDUS DOG[1]

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The only obvious abnormality of the diabetes insipidus animal is its excessive excretion of water; otherwise the renal function of such an animal, insofar as it has been studied (19, 25), is apparently normal. The question, however, remains, whether this inability to reabsorb water is the fundamental deficiency of the diabetes insipidus renal tubule, or whether it is merely secondary to some alteration in the tubular reabsorption of solutes. The possibility of a disturbance in chloride excretion has been investigated many times, but the continuation of these experiments indicates that no decisive answer has been obtained. This lack of success was probably due to the concentration of most investigators on excretion alone, and it is extremely difficult to evaluate their data, since excretion is the net result of at least two variables, glomerular filtration and tubular reabsorption.

Some electrolytes are reabsorbed like glucose from the glomerular filtrate in definitely limited quantities. If the glomeruli, because of increased volume of filtrate or of an elevated plasma concentration, deliver such a substance to the tubules in an amount greater than the tubular capacity for reabsorption, the excess is excreted in the urine; phosphates and sulphates belong to this group. The maximal rate of tubular reabsorption of phosphate in the dog is 0.8 to 4.2 mgm./min., but is dependent upon the acid-base balance of the animal (9), and is subject to vitamin and hormonal control (10). Goudsmit, Power and Bollman (7) showed that sulphate clearances asymptotically approach the simultaneous creatinine clearances in dogs as the plasma sulphate is progressively increased. This indicates a low tubular maximum of sulphate reabsorption, and a recalculation of their data reveals the maximum to be less than 10 mgm./min.

A second type of renal reabsorption is comparable to the intestinal reabsorption of salt solution, in which the volume of fluid and the quantity of electrolyte transferred are closely related. When a solution of sodium chloride is placed in a loop of gut, the salt is absorbed along with most of the water, until a chloride-free fluid is left in the intestinal lumen. Visscher (22) has pointed out the similarity of this process to the renal tubular reabsorption of electrolyte, where the parallel is especially close during water diuresis and in diabetes insipidus. If the disturbance in water reabsorption in diabetes insipidus is secondary to an abnormal electrolyte reabsorption, or more specifically chloride reabsorption, it is probable that a mechanism of this kind is involved.

Our primary problem was to establish the behavior of chloride in the normal kidney, since this is extremely ill-defined and is essential to any hypothesis of an

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abnormal process in diabetes insipidus. We soon found that there was no apparent limit either to the amount of chloride excreted per minute or to its concentration in the excreted urine. We were also unable to establish any general relationship between chloride excretion and plasma chloride values. When we turned our attention to reabsorption, we found that there was no apparent tubular maximum for chloride, or, if there was such a limit, it was not attained when the quantity of chloride filtered was increased as much as fourfold. If, however, the quantity of chloride reabsorbed was compared with the quantity of water reabsorbed in the same time, a very definite relationship was apparent, and one which was consistent from experiment to experiment and from one dog to another.

This relationship, the ratio of reabsorbed chloride to reabsorbed water, is not intended to express anything as simple as the concentration of the reabsorbed solution. Walker et al. (23), in studies on single mammalian nephrons, have shown that the glomerular fluid soon attains, as it progresses down the tubule, a chloride concentration about 1.4 times that of plasma. Therefore, chloride and water seem to be reabsorbed in different parts of the tubule, and the ratio of reabsorbed chloride to water is merely the resultant of these reabsorptive processes. This ratio is not a constant, but is related to changes in plasma chloride, and it was soon found that a comparison of the chloride:water ratio in the reabsorbate with the chloride concentration of the plasma was a useful expression of the reabsorptive process; it was designated the chloride R/P.

When saline solutions of different concentrations were infused at the same rate into a normal dog in successive experiments, it was found that a family of curves resulted from relating the chloride R/P to time. The configuration of each curve was characteristic of the concentration of the saline infused. When the experiments were repeated on a dog with diabetes insipidus, the responses were unpredictable and not related to the concentration of the injected fluid. The chloride R/P of the diabetes insipidus dog is initially greater than one and may rise considerably further during saline infusion, reaching a maximum value in our experiments of 1.2. Since the addition of Pitressin to the infusion fluid altered these responses until they approximated those of the normal animal, it was concluded that the neurohypophysis regulates the partition of water between tubular reabsorbate and urine. This is true, of course, only of that fraction of the water, about 20 per cent, which is not reabsorbed by the diabetes insipidus tubule; the remaining 80 per cent of water filtered is reabsorbed by the tubule even in the complete absence of the posterior lobe. Our conclusion is based on data obtained from more than one hundred experiments on three normal dogs and four dogs with experimental diabetes insipidus.

PROCEDURES. Experimental diabetes insipidus was produced by transection of the pituitary stalk. Our usual procedure (8) for creatinine clearances was followed, with these modifications: venous blood was drawn under oil into oxalated tubes for centrifugation; and fluids, other than water by stomach tube, were administered intravenously with a constant infusion pump. In the earlier experiments, plasma and urine chlorides were determined by the method of Van Slyke and McLean (16), but later the simpler mercurimetric titration (18) was

adopted, after it had been established that analyses by the two methods were almost identical. Glucose, in tungstic acid filtrates of plasma and in diluted aliquots of urine, was determined by measuring with a photoelectric colorimeter the reduction of an alkaline ferricyanide solution.

Calculations. The volume of fluid reabsorbed per minute by the tubules was calculated by subtracting the urine flow in cubic centimeter per minute from the creatinine clearance; reabsorbed chloride by subtracting the quantity excreted per minute from the quantity filtered. This last value was obtained by multiplying the volume of glomerular filtrate by the milligrams of chloride per cubic centimeter of plasma. The ratio of chloride to water in the reabsorbate, $\frac{\text{mgm. Cl}}{\text{cc. } H_2O} \times 100 =$ mgm. per cent reabsorbed, was then compared to the plasma chloride according to the equation: Chloride $R/P = \frac{\text{mgm. per cent reabsorbed Cl}}{\text{mgm. per cent plasma Cl}}$.

The correction factor for the solid content of plasma has not been applied in these calculations, since it did not significantly affect the chloride R/P value.

RESULTS. The earlier experiments were performed to obtain data for analysis in the hope of revealing factors which limited chloride excretion or reabsorption. They were done, therefore, on normal dogs and included water diuresis, intravenous infusion of glucose and hypo-, iso- and hypertonic saline solutions. Three of these experiments, in which different concentrations of saline were infused, are presented in table 1. These data have been analysed in several ways. The lack of correlation between urine flow and chloride excretion is obvious on inspection of table 1. However, a nearly linear relationship exists between the quantity of reabsorbed water and of reabsorbed chloride (fig. 1). When 10 per cent glucose or 0.2 to 0.3 per cent NaCl solutions were infused, and the plasma chloride concentration lowered, the curve was altered as less chloride was reabsorbed per unit volume of water (fig. 1). If the plasma chloride concentration is greatly elevated by the infusion of 2.0 to 5.0 per cent NaCl, the chloride:water ratio of the tubular reabsorbate is definitely increased (fig. 1). This deviation of the chloride:water ratio at low and high plasma concentrations indicates its dependence upon changes in the salt content of the plasma. The chloride R/P was found to change during the course of each experiment in which hypo- or strongly hypertonic saline solutions were given. When the data in table 1 are analysed to show the temporal course of the chloride R/P, three distinct curves are obtained (fig. 2). While it appears from this figure that the contour of each curve is related to the concentration of the salt solution injected, other variables, such as the rate and duration of the infusion, are not excluded as influencing factors. Six infusions into the same normal dog, L, were constant except for the concentration of the solution, which was 0.55, 0.67, 1.06, 1.5, 2.0 and 2.5 per cent NaCl. When the chloride R/P is related to time (fig. 3), a progressive modification of the response with increasing concentrations of NaCl is apparent.

The next part of this investigation is concerned with the regulating mechanism which so exactly controlled the partition of water between urine and reabsorbate that when hypotonic solutions were given, little salt and large volumes of water

WATER REABSORPTION CC/MIN.

were excreted, and when strong saline was infused, large quantities of salt were excreted in highly concentrated urine. The neurohypophysis was naturally given first consideration.

When the subject was a dog with diabetes insipidus, the results were in sharp contrast to the highly integrated responses of the normal dog. Infusions of 2.0 or 2.5 per cent NaCl instead of producing a depression of the chloride R/P, often caused a further rise of the already elevated ratio (fig. 4). The posterior lobe of the pituitary, therefore, seems to be involved in the depression of the chloride

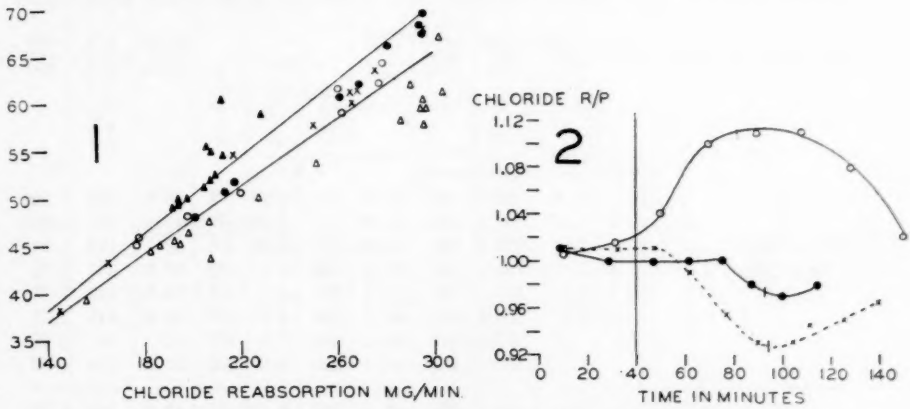


Fig. 1. Chloride reabsorption plotted against water reabsorption. Those data which are taken from table 1 are represented by the following symbols: open circles, 0.5 per cent NaCl; solid circles, 0.685 per cent NaCl; crosses, 2.0 per cent NaCl. Solid triangles represent the data from experiments in which the plasma chloride was reduced by the infusion of 0.2 to 0.3 per cent NaCl or of glucose solutions. Open triangles are data obtained during or after the infusion of 2.5 to 5.0 per cent NaCl solutions when the plasma chloride concentration was greatly elevated.

Fig. 2. Temporal course of the chloride R/P on infusion of 0.5 per cent (open circles), 0.685 per cent (solid circles), and 2.0 per cent NaCl (crosses). Beginning of infusions at 40 minutes is indicated by long vertical line; end of infusions by short vertical lines. Data from table 1.

R/P below one. Attempts to convert the response of the diabetes insipidus dog to normal by substitution therapy with Pitressin (Parke, Davis & Co.) were successful. In a series of infusion experiments, repeated at weekly intervals, 2.5 per cent NaCl was injected at 10 cc./min. for 45 minutes. In the first no Pitressin was given; in the others Pitressin was added to the saline so that it was administered at rates of 60, 180 and 720 milliunits per hour. The results are given in table 2, and the chloride R/P plotted against time in figure 4. While the depression of the chloride R/P with 720 mu./hr. is about as great as that seen in experiments on normal dogs (see fig. 3, lowest curve) receiving the same infusion, there are minor differences in the responses. The response of the normal dog has a latent period of 15 to 30 minutes, measured from the beginning of the infusion, and continues for at least 30 minutes afterward. In the dia-

betes insipidus dog receiving Pitressin, the response begins with the infusion and, at the end of the infusion, falls off in every case more rapidly than the normal, but at a rate which is decreased as the Pitressin dose is increased. The

TABLE 1

Renal response of the normal dog to the infusion of hypo-, iso- and hypertonic sodium chloride solutions

DOG AND INFUSION	PERIOD	DURATION	URINE FLOW	URINE CHLORIDE	PLASMA CHLORIDE	CREATININE CLEARANCE	CHLORIDE (MGM./MIN.)			WATER REABSORBED	MGM. CHLORIDE REABSORBED 100 CC. WATER REABSORBED	CHLORIDE R/P
							Filtered	Excreted	Reabsorbed			
		Minutes	*cc./min.*	*mgm.%*	*mgm.%*					*cc./min.*		
P	1	20	0.56	31.9	385	46.5	178	0.18	178	45.9	388	1.006
	2	20.2	0.73	24.3	385	45.9	177	0.18	177	45.2	391	1.015
0.5% NaCl	3	21.0	2.91	92.5	395	51.0	201	2.70	198	48	413	1.04
29 cc./min.	4	20.2	9.12	155.0	393	59.7	235	15.0	220	50.6	434	1.10
	5	20.4	10.6	134	390	75.0	292	14.2	278	64.4	432	1.11
	6	17.8	8.4	79.5	400	67.3	269	6.7	262	58.9	445	1.11
	7	20.0	7.2	97.0	411	69.0	284	7.0	277	62	447	1.08
	8	21.1	3.5	279	415	65.0	270	9.8	260	61.5	422	1.02
P	1	28.6	0.3	43.3	379	43.5	165	0.13	165	43.2	383	1.01
	2	16.2	0.35	22.4	379	38.3	145	0.08	145	38.0	382	1.01
	3	14.0	0.99	100	395	55.4	218	0.99	217	54.4	399	1.01
2% NaCl,	4	15.5	2.67	501	427	54.6	233	13.4	220	51.9	424	0.99
8.7 cc./min.	5	14.6	5.73	672	454	63.5	288	38.5	250	57.8	433	0.955
	6	9.9	7.95	736	478	68.0	325	58.6	266	60.0	443	0.93
	7	19.9	5.28	874	470	66.7	314	46.1	268	61.4	438	0.93
	8	14.1	3.12	982	461	70.8	326	30.6	295	67.7	436	0.945
	9	14.4	2.47	1050	456	66.0	301	26.0	275	63.5	433	0.95
	10	14.3	2.13	930	450	63.5	285	19.8	265	61.3	433	0.965
E	1	15.3	0.67	179	415	51.5	214	1.2	213	50.8	419	1.01
	2	14.9	0.63	199	415	52.5	218	1.2	217	51.9	418	1.01
	3	24.4	0.51	200	415	48.5	201	1.0	200	48.0	417	1.00
	4	13.8	0.79	350	429	61.7	264	2.8	261	60.9	429	1.00
0.685% NaCl,	5	14.1	1.30	522	431	63.6	275	6.8	268	62.3	430	1.00
21.5 cc./min.	6	13.0	3.00	626	437	70.6	309	14.4	295	67.6	436	1.00
	7	11.7	3.68	605	436	72.3	315	22.2	293	68.6	428	0.98
	8	14.0	3.43	668	433	70.0	303	22.9	280	66.6	421	0.97
	9	14.0	2.78	688	433	72.5	314	19.1	295	69.7	424	0.98

continued fall of the chloride R/P in the normal dog suggests a continued liberation of the antidiuretic hormone.

During the infusion of 2.5 per cent NaCl and Pitressin, the creatinine U/P falls, even when as much as 720 mu./hr. is injected. This behavior of the cre-

atinine U/P is the opposite of that seen when the antidiuretic hormone is given during a water diuresis or to an animal with diabetes insipidus. Under these conditions an elevation of the creatinine U/P, signifying a greater tubular reabsorption of water, is invariably obtained, and Hickey, Hare and Hare (11) have demonstrated a quantitative relationship between the dose of pituitrin and the response. From the present experiments it is clear that changes in the creatinine U/P during a saline diuresis, whether in a normal dog (fig. 6) or in a diabetes insipidus dog receiving Pitressin (table 2), have no significance and are valueless as indicators of the action of the antidiuretic hormone. In contrast to

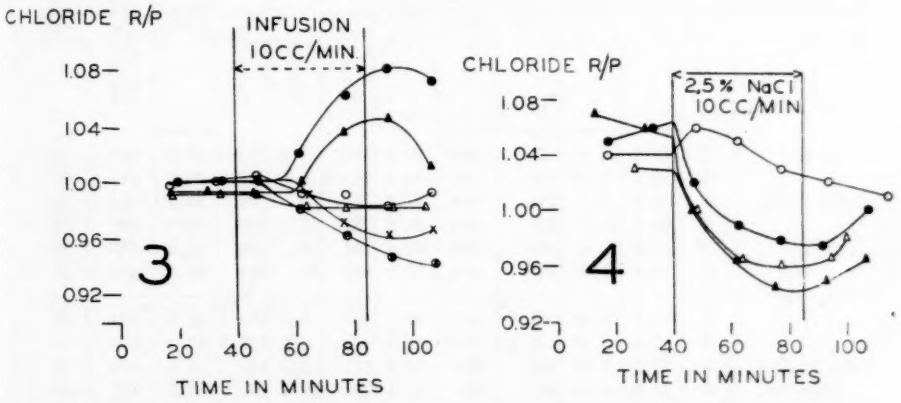


Fig. 3. Temporal course of the chloride R/P during infusions of graded saline solutions into the same normal dog, at 10 cc./min. for 45 minutes. Solid circles, 0.55 per cent; solid triangles, 0.67 per cent; open triangles, 1.06 per cent; open circles, 1.5 per cent; crosses, 2.0 per cent; and enclosed crosses, 2.5 per cent NaCl.

Fig. 4. Relationship of the chloride R/P of a dog (S) with diabetes insipidus to the amount of Pitressin added to the infusion fluid (2.5 per cent NaCl). The vertical lines enclose the infusion period. Open circles, no Pitressin; solid circles, 60 mu/hr.; open triangles, 180 mu/hr.; solid triangles, 720 mu/hr. Data from table 2.

this, the chloride R/P (fig. 4) is related to the amount of antidiuretic hormone introduced into the dog's circulation during a saline diuresis.

An elevation of the chloride R/P is the permanent effect of the destruction of the pars nervosa of the pituitary and the temporary consequence of the ingestion of water or the injection of glucose or hypotonic saline. It seemed probable that these procedures elevated the chloride R/P by inhibiting the liberation of the posterior lobe hormone. If this were true, the administration of small amounts of the hormone should decrease or prevent the elevation of the chloride R/P during the course of such a diuresis. This has been tested repeatedly, and the experiment selected for presentation illustrates the effect of 1.7 and 6.8 mu. of Pitressin per hour on the diuresis resulting from the injection of 0.55 per cent NaCl at 10 cc./min. (fig. 5). The same normal dog, L, was the subject for all three experiments which were as nearly alike as possible. The response of the

animal to hypotonic saline, as indicated by the chloride R/P, is measurably depressed by 1.7 mu. of Pitressin per hour, and completely suppressed by 6.8 mu./hr. The minute quantity of the antidiuretic hormone needed to restore

TABLE 2

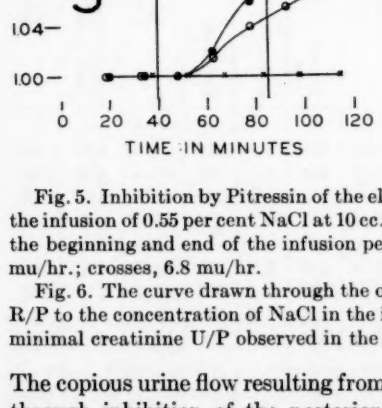
The effect of graded doses of Pitressin on the renal excretion of sodium chloride and water by a dog with diabetes insipidus

DOG AND INFUSION	PERIOD	DURATION	URINE FLOW	URINE CHLORIDE	PLASMA CHLORIDE	CREATININE CLEARANCE	CHLORIDE (MGM./MIN.)			WATER REABSORBED	MGM. CHLORIDE REABSORBED/100 CC. WATER REABSORBED	CHLORIDE R/P
							Filtered	Excreted	Reabsorbed			
		minutes	*cc./min.*	*mgm.%*	*mgm.%*					*cc./min.*		
S	1	47.1	2.03	59	384	39.9	153	1.2	152	37.9	402	1.04
2.5% NaCl	2	15.2	3.96	205	420	43.2	181.5	8.1	173.4	39.2	445	1.06
	3	15.4	8.4	345	448	47.6	213	29	184	39.2	469	1.05
	4	14.4	13.5	423	475	59.5	282	57	225	46.0	489	1.03
	5	18.0	10.6	435	478	52.6	251	46	205	42.0	488	1.02
	6	18.7	7.24	441	473	52.2	247	32	215	45.0	478	1.01
S	1	14.7	2.58	23.7	382	43.8	167	0.6	166.4	41.2	403	1.05
	2	15.5	2.28	21.4	379	39.3	149	0.5	148.5	37.0	402	1.06
2.5% NaCl + Pitressin, 60 mu/hr.	3	15.2	1.96	265	396	43.6	173	5.2	168	41.6	404	1.02
	4	14.3	6.05	467	431	51.5	222	28.3	194	45.5	427	0.99
	5	15.1	12.4	491	458	60.4	277	60.6	216	48.0	450	0.98
	6	14.0	11.4	514	459	60.6	278	58.5	220	49.2	445	0.975
	7	15.1	7.7	460	448	58.1	261	35.2	226	50.4	448	1.00
S	1	27.4	1.54	66.5	388	34.4	133	1.0	132	32.8	403	1.04
2.5% NaCl + Pitressin, 180 mu/hr.	2	14.0	1.21	400	424	39.6	168	4.85	163	38.4	424	1.00
	3	15.6	4.18	673	460	47.2	217	26.0	191	43.0	444	0.965
	4	14.7	10.85	564	484	56.1	272	61.3	211	45.2	466	0.965
	5	19.7	11.40	566	481	60.6	292	65.0	227	49.2	462	0.96
	6	10.9	8.05	538	471	60.5	285	43.2	242	52.5	461	0.98
S	1	14.8	3.5	68.5	371	41.6	154	1.95	152	38.1	399	1.07
	2	20.7	2.8	63.5	372	42.2	157	1.8	155	39.4	394	1.06
2.5% NaCl + Pitressin, 720 mu/hr.	3	13.7	0.73	425	396	40.1	159	3.1	156	39.4	396	1.00
	4	14.7	2.0	770	445	45.6	203	15.5	187.5	43.6	430	0.965
	5	13.3	7.6	625	467	53.4	249	47.4	201.6	45.8	442	0.945
	6	15.0	10.9	567	466	58.4	272	61.5	210.5	47.5	444	0.95
	7	15.5	9.1	541	458	58.8	269	49.4	219.6	49.7	442	0.965

and maintain a chloride R/P of unity is again demonstrated in an experiment on a dog with diabetes insipidus. A 1.5 per cent solution of NaCl was infused for 80 minutes at 5 cc./min.; later the experiment was repeated with the addition of Pitressin to the saline so that its infusion rate was 4.25 mu./hr. The results

summarized in table 3 further justify the hypothesis that an elevated chloride R/P is evidence of inhibition of the neurohypophysis.

An application of this thesis, that changes in the chloride R/P reflect changes in the rate of release of the antidiuretic hormone, to a further analysis of the data from the normal dog receiving different concentrations of saline (fig. 3), permits an interpretation of the diuretic action of salt solutions. When the concentration of each saline solution is plotted against its diuretic effectiveness expressed by the minimal creatinine U/P obtained during or after the infusion of the solution, it is clear that very dilute or very concentrated saline are equally effective diuretics (fig. 6). When the maximal change of the chloride R/P is similarly plotted against the salt content of the infusion fluid, different explanations for the diuresis with dilute and with concentrated saline are suggested.



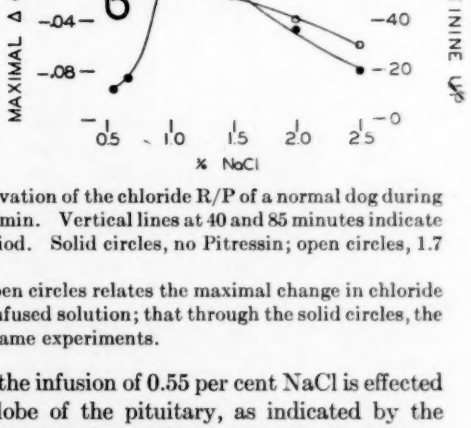


Fig. 5. Inhibition by Pitressin of the elevation of the chloride R/P of a normal dog during the infusion of 0.55 per cent NaCl at 10 cc./min. Vertical lines at 40 and 85 minutes indicate the beginning and end of the infusion period. Solid circles, no Pitressin; open circles, 1.7 mu/hr.; crosses, 6.8 mu/hr.

Fig. 6. The curve drawn through the open circles relates the maximal change in chloride R/P to the concentration of NaCl in the infused solution; that through the solid circles, the minimal creatinine U/P observed in the same experiments.

The copious urine flow resulting from the infusion of 0.55 per cent NaCl is effected through inhibition of the posterior lobe of the pituitary, as indicated by the marked elevation of the chloride R/P. But during the comparable diuresis obtained with 2.5 per cent NaCl, where the depression in the chloride R/P suggests the release of several hundred milliunits of antidiuretic hormone, the volume of urine excreted can be attributed to the large amount of salt excreted. The infusion of 0.9 to 1.5 per cent NaCl neither inhibits the pituitary nor leads to the excretion of sufficient salt to produce an osmotic diuresis; solutions within this range of concentrations have, therefore, the least diuretic effect.

So far, we have centered attention on the changes in the partition of water between urine and tubular reabsorbate and the hypophyseal regulation of this distribution. Since the nature of the calculation for the derivation of the chloride R/P is such that a change in the ratio would be produced by a change in the tubular reabsorption of chloride, we shall now consider this process and the

evidence for its control by the pituitary. When we injected 0.1 to 2.0 mu. of Pitressin into dogs with diabetes insipidus, we regularly obtained a marked decrease in urine flow, but no increase of chloride excretion was observed in any of the ten experiments. Furthermore, we did find the chloride excretion increased three- to fivefold in a normal dog during water diuresis, when the antidiuretic content of the blood is assumed to be subnormal.

Our experiments on dogs with diabetes insipidus given a constant infusion of 2.5 per cent NaCl solution with graded doses of Pitressin dissociate antidiuretic activity from any demonstrable change in chloride excretion. When the rate of chloride excretion during each experiment is related to time, no significant or consistent variation appears which can be related to the amount of Pitressin

TABLE 3

The control of a saline diuresis in a dog with diabetes insipidus with a small dose of Pitressin

DOG AND INFUSION	PERIOD	DURATION	URINE FLOW	URINE CHLORIDE	PLASMA CHLORIDE	CREATININE CLEARANCE	CHLORIDE (MGM./MIN.)			WATER REABSORBED	MGM. CHLORIDE REABSORBED / 100 CC. WATER REABSORBED	CHLORIDE R/P
							Filtered	Excreted	Reabsorbed			
		minutes	*cc./min.*	*mgm.%*	*mgm.%*					*cc./min.*		
D 1.5% NaCl, 5 cc./min.	1	20.0	5.01	39.4	400	79.5	318	2	316	74.5	424	1.06
	2	19.6	6.21	128	406	84.0	341	8	333	77.8	428	1.05
	3	20.9	8.22	187	410	86.0	353	15.3	338	77.8	434	1.06
	4	19.7	11.0	181	427	79.8	342	20	322	68.8	468	1.09
D 1.5% NaCl, 5 cc./min., Pitressin, 4.25 mu/hr.	1	19.5	1.85	50	397	60.4	239	0.9	238	58.5	407	1.02
	2	19.0	2.06	295	420	69.8	293	6.0	287	67.7	424	1.01
	3	21.2	3.74	442	430	75.8	326	16.5	310	72.1	430	1.00
	4	21.1	5.45	462	444	75.4	334	25	309	70.0	442	0.995

injected (fig. 7); the amount of water excreted is, however, clearly dependent (fig. 8). Additional evidence is included in table 3, where marked antidiuresis was obtained without any such change in chloride excretion. For two reasons these observations are of only limited value as support for the thesis that physiological quantities of the antidiuretic hormone do not act by inhibiting tubular reabsorption of chloride. In the first place, Pitressin, a fraction of pituitary extract, was used, and Kuschinsky and Bundschuh (22) find the principle of pituitrin affecting chloride excretion associated with the oxytocic rather than with the antidiuretic fraction. A second objection is that the dogs were heavily loaded with salt. However, the observations do justify the contention that an antidiuresis can be produced by increasing only the reabsorption of water. Further support for this idea is found in experiments on the diabetes insipidus dog

with a normal salt intake, where Pitressin greatly facilitated the tubular reabsorption of water without affecting chloride excretion.

White and Findley (24) found that patients with diabetes insipidus failed to excrete an oral dose of salt as rapidly as normal persons, and that pituitrin therapy was not effective in correcting this derangement. When we followed the chloride excretion before, during and after similar injections of salt into normal and diabetes insipidus dogs, no consistent difference between the two groups was revealed. On several occasions the polyuric dog excreted more salt than the

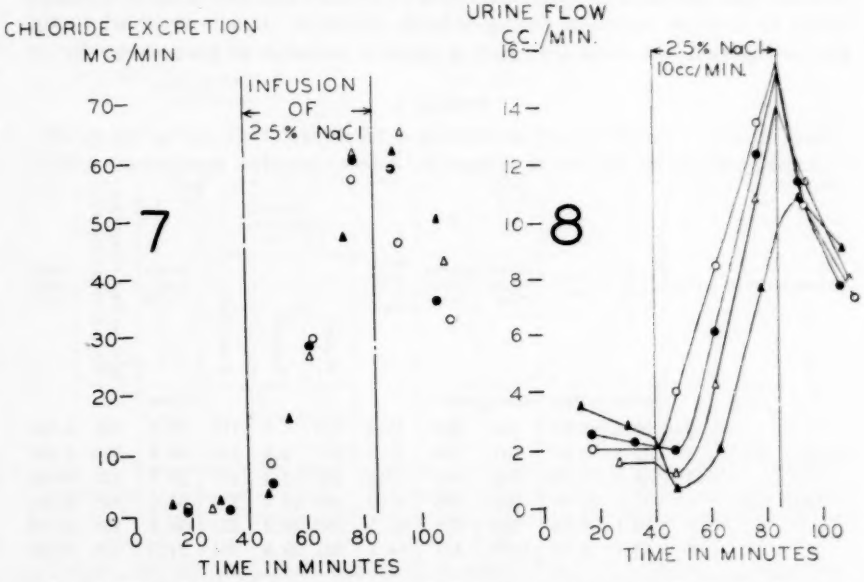


Fig. 7. Independence of chloride excretion of Pitressin administration in a dog (S) with diabetes insipidus. Vertical lines indicate period of infusion of 2.5 per cent NaCl at 10 cc./min. Open circles, no Pitressin; solid circles, 60 mu/hr.; open triangles, 180 mu/hr.; solid triangles, 720 mu/hr. Data from table 2.

Fig. 8. Antidiuretic effect of Pitressin during saline diuresis in a dog (S) with diabetes insipidus. Symbols the same as in figure 7.

normal control. One of these comparisons is presented in detail in table 4 and the first experiment of table 2. The excretion of chloride, plotted against time in figure 9, is greater in the polyuric dog in spite of the fact that glomerular filtration is less. The more rapid rise of the plasma concentration in the diabetes insipidus dogs has been seen repeatedly, and probably has its origin in the excessive water loss of these animals. In this instance the polyuric dog excreted 744 cc. of urine during the experiment which lasted an hour and a half, while the normal dog excreted only 185 cc. during the same time. Probably for the same reason, the polyuric dog developed a severe salt intoxication (26) with tremors, weakness and vomiting. However, the higher rate of salt excretion by the poly-

uric dog has been observed in five experiments of this type when no symptoms of salt intoxication were apparent.

Water diuresis frequently (17, 21), and glucose (2) or sucrose (12) regularly increase chloride excretion. An infusion of 10 per cent glucose at 10 cc./min. may cause an initial tenfold increase in the renal loss of salt (table 5 and fig. 10), but this effect falls off, even during the first few hours, so that the chloride excretion rate becomes only two or three times normal. This elevated excretion occurs during a progressive fall of plasma chloride, and therefore does not have its origin in the animal's chloride load, which Wolf (26) has shown to be a related factor in chloride excretion during chloride infusion. If salt is added to the glucose solution, the renal excretion of chloride proceeds at least as rapidly, or

TABLE 4

Renal response of a normal dog to the infusion of 2.5 per cent NaCl at 10 cc. per minute

Compare with similar experiment of dog with diabetes insipidus, experiment 1, table 2

DOG AND INFUSION	PERIOD	DURATION	URINE FLOW	URINE CHLORIDE	PLASMA CHLORIDE	CREATININE CLEARANCE	CHLORIDE (MGM./MIN.)			WATER REABSORBED	MGM. CHLORIDE REABSORBED / 100 CC. WATER REABSORBED	CHLORIDE R/P
							Filtered	Excreted	Reabsorbed			
		minutes	*cc./min.*	*mgm.%*	*mgm.%*					*cc./min.*		
L	1	16.4	0.47	245	389	45.8	178	1.1	177	45.3	391	1.00
Normal, 2.5%	2	11.5	0.82	341	399	47.0	187	2.8	184	46.2	398	1.00
NaCl,	3	16.8	1.79	742	432	52.5	227	13.3	214	50.7	422	0.98
10 cc./min.	4	15.2	2.96	860	458	57.6	264	25.4	239	54.6	438	0.96
	5	16.1	3.12	934	459	61.5	282	29.2	253	58.4	434	0.945
	6	15.1	2.78	1070	454	64.1	291	29.8	261	61.3	426	0.94

even more rapidly than when the same amount of salt is given alone (table 5 and fig. 10). This occurs even when the plasma chloride is still below normal.

Perhaps the most consistent effect of the administration of salt to a dog, whether by mouth or by intravenous injection, is an increase in the rate of glomerular filtration (19). While it is true that the infusion of a large volume of water as a vehicle for glucose (20) may cause an increase in glomerular filtration, the change in our experiments has never been as great as that caused by the infusion of sodium chloride. Furthermore, when 10 per cent glucose has been infused at a constant rate for an hour or more, and the elevated rate of filtration has become stabilized, the addition of salt to the infusion fluid caused a further and sustained rise. This is illustrated in the first experiment of table 5.

DISCUSSION. Walker's (23) studies on the tubular reabsorption of chloride are, unfortunately, incomplete and limited to the proximal convoluted tubule. Until these direct studies have been extended to include the rest of the nephron

and the hormonal factors which regulate reabsorption, only the final result of the various tubular processes can be determined from experiments on the intact animal. The proper choice of an electrolyte or other substance to be associated with the tubular reabsorption of water in the calculation of R/P ratios can be made, of course, only after the tubular transfer of that substance is clearly shown to be an essential process in water reabsorption. The choice of chloride was not made on that basis, and may, therefore, be premature. But the use of the chloride R/P does permit some insight into the response of the normal dog kidney to endogenous antidiuretic hormone, for the temporal course of the chloride R/P

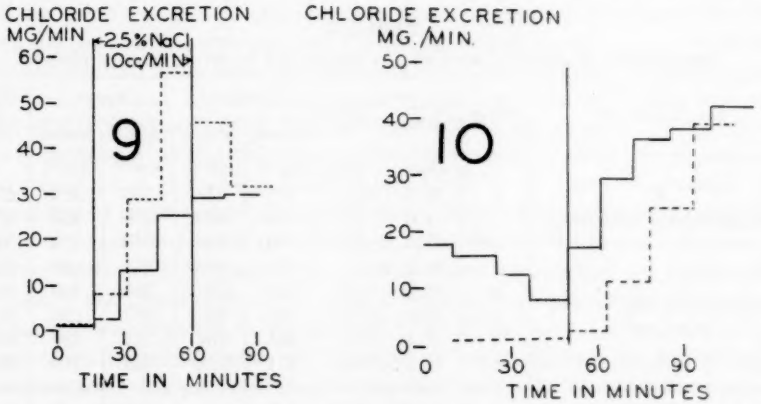


Fig. 9. Comparison of the renal excretion of chloride by a normal (solid line) and a diabetes insipidus (broken line) dog. The vertical lines indicate the infusion of 2.5 per cent NaCl at 10 cc./min. Data for normal dog from table 4; for diabetes insipidus dog from expt. 1, table 2.

Fig. 10. Facilitation of chloride excretion by glucose diuresis. To the left of the vertical line, the excretion of chloride by a normal dog during glucose infusion (solid line) begun two hours before zero time is compared with the basal normal chloride excretion (broken line). At the vertical line, the infusion of 2 per cent saline was begun in both cases; the NaCl was added to the glucose solution so that in both cases the infusion rate was 10 cc./min.

during a saline diuresis has been a reliable indicator of the amount of the hormone discharged by the pituitary or introduced into the circulation by infusion.

Gilman and Goodman (5) discovered that the high rate of urine flow of a normal animal after the administration of 5 per cent NaCl is attended by a greatly augmented liberation and renal excretion of the antidiuretic hormone. They estimated that 8 to 12 rats excreted 100 to 200 milliunits within six hours after being given 5 per cent of their body weight of salt solution by stomach tube. Ingram, Ladd and Benbow (13), using the same methods, found that the antidiuretic material excreted by cats during saline diuresis originated in the pars nervosa of the pituitary. According to their assays, 20 to 420 milliunits per day were excreted by a cat, dehydrated by intravenous injection of 10 per cent NaCl after purging with $MgSO_4$ by stomach tube. We have estimated that a

normal dog of 15 kgm. liberates into its blood stream about one unit of pituitrin when given 450 cc. of 2.5 per cent NaCl by intravenous infusion (figs. 3 and 4). Our data are not comparable in a quantitative manner with those mentioned above, since the latter measured the hormone excreted by a normal animal, while we have measured replacements necessary to reproduce a response in a dog deprived of its source of the hormone. All the data are in agreement, however, that saline, in hypertonic solutions, is extremely effective in causing a release of pituitrin; whether this is a specific effect of NaCl, or simply dehydration, is not clear (11). This excessive liberation of the hormone may well account for the failure of huge doses to inhibit a saline diuresis. Goodman and

TABLE 5

Augmentation of chloride excretion of a normal dog by the infusion of glucose

DOG AND INFUSION	PERIOD	DURATION	URINE FLOW	URINE		PLASMA		CREATININE CLEARANCE
				Chloride	Glucose	Chloride	Glucose	
		minutes	*cc./min.*	*mgm.%*	*mgm.%*	*mgm.%*	*mgm.%*	
E	1	14.9	12.2	127	3000	355	910	57.1
*10% glucose at 10 cc./min.	2	11.6	7.6	158	4160	355	850	57.6
	3	13.3	5.5	136	5000	350	710	59.6
	4	11.7	7.7	216	4450	355	800	69.0
10% glucose + 2% NaCl at 10 cc./min.	5	11.8	11.1	262	3270	365	840	66.6
	6	12.8	11.5	313	2940	390	830	68.8
	7	14.1	10.4	361	3160	415	780	67.4
	8	14.2	10.2	411	3160	425	710	69.8
E	1	14.2	0.21	346		395		50.2
	2	11.3	0.23	350		398		48.1
	3	13.5	0.47	493		396		50.2
2% NaCl at 10 cc./min.	4	14.6	1.43	766		422		70.4
	5	16.4	2.69	884		438		66.0
	6	14.2	4.82	804		455		66.8
	7	15.8	6.20	716		465		73.4
	8	14.4	6.08	640		453		72.3

* The glucose infusion was started 2 hours before the beginning of the first period.

Gilman (6) showed that one unit of Pitressin failed to inhibit the diuresis resulting from the injection of 10 per cent saline, while doses $\frac{1}{200}$ as large will sharply depress a water diuresis.

Strong solutions of NaCl may cause the release of the antidiuretic hormone by some direct action on the posterior lobe of the pituitary, or through a reflex originating in some chemoreceptor, or, more probably, through stimulation of the cells of the highly vascular supra-optic nuclei, whose processes pass down the pituitary stalk to the neurohypophysis. Excision of the pituitary or transection of the pituitary stalk with subsequent atrophy of the posterior lobe abolishes the response, but this shows only that the antidiuretic hormone originates in the neurohypophysis, and throws no light on the mechanism of its

release. Sensitivity of the neurones in the supra-optic nuclei to changes in the chemical composition of the blood has been suggested by Craigie (4) and by Ingram, Ladd and Benbow (13), but direct evidence, such as might be obtained by recording action potentials from the nuclei or the pituitary stalk during the infusion of different solutions, has not been provided. Nor have the changes in the blood which cause the pituitary to liberate or withhold the antidiuretic hormone been defined. It may be that rate of change is more important than magnitude, as has been suggested by Baldes and Smirk (3), who investigated the rôle of the osmotic pressure of the plasma in initiating a water diuresis.

MacKay and MacKay (15) and Aitken (1) have lowered the plasma chloride until the urine was chloride-free by urea diuresis, salt-free diets, and sweating. When the lost chloride was replaced, the plasma concentration at which chloride reappeared in the urine was designated the chloride threshold. Aitken's objections to the method, which failed to give reproducible results, led him to recommend that the concept of a chloride threshold be abandoned. In Rehberg's (17) observations on himself, plasma chloride was changed by the ingestion of salt or water, and glomerular filtration measured by creatinine clearances. His calculations of reabsorption of chloride reveal that there is no tubular maximum. However, when the percentage of filtered chloride excreted in the urine was related to creatinine U/P, two curves were obtained representing the results when the plasma chloride was above or below 375 mgm. per cent. This led him to set this plasma concentration as the chloride threshold in man. Of far more interest to us was his finding that after the ingestion of water, chloride was reabsorbed in a concentration higher than that of plasma, while after the ingestion of salt, this relationship was reversed.

In our experiments on dogs, the rate of tubular reabsorption of chloride has been as high as 500 mgm./min., and this upper limit seems to have been imposed by the tolerance of the animal for hypertonic saline, rather than by the inability of the tubules to transfer more chloride back into the blood. Attempts to find a plasma chloride concentration at which significant quantities of chloride began to be excreted were unsuccessful, and determinations of the threshold could not be repeated using the same subject and experimental procedures. When the conditions of the experiments were changed, the threshold varied so widely that it lost all significance. For example, during the glucose diuresis, chloride excretion may proceed at about 50 mgm./min., even though the plasma chloride falls 50 to 75 mgm. below the preinfusion level, and when glomerular filtration is only slightly increased. Since a urea (15) or sucrose (12) diuresis causes a similar chloride excretion at low plasma concentrations, it seems that an osmotic diuresis disturbs tubular reabsorption of chloride. When saline, instead of glucose, was infused, chloride excretion was invariably increased, regardless of the preinfusion plasma chloride concentration or the rate of glomerular filtration. This has been true even when the saline was so dilute (0.2–0.4 per cent NaCl) that the plasma chloride concentration was diminished. On the basis of these data, it is obviously impossible to define a chloride threshold for the dog.

SUMMARY

The renal tubular reabsorption and the urinary excretion of water and of chloride have been studied in normal and diabetes insipidus dogs during water, saline and glucose diureses. The factors which determine the partition of chloride between tubular reabsorbate and urine are not revealed, but it is shown that there is neither a maximal rate of tubular reabsorption nor a renal threshold for chloride. Pitressin does not inhibit its tubular reabsorption nor facilitate its excretion. Renal tubular reabsorption of water is related to the reabsorption of chloride under all conditions of these experiments, and the antidiuretic hormone controls this relationship by regulating water reabsorption.

Hypertonic saline stimulates, while hypotonic saline, glucose solutions, and water inhibit the secretion of antidiuretic hormone. It is proposed that for the regulation of water exchange there is a system which includes the hypothalamico-hypophyseal mechanism, which, through its sensitivity to changes in the salt content of the blood, controls the secretion of pituitrin, and thereby the concentration in which salt is reabsorbed by the renal tubules back into the blood.

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INCREASED RED BLOOD CELL FRAGILITY AFTER FAT INGESTION[1]

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There have been indications that ingested fat might be injurious to red blood cells. In studies on relatively few human individuals over brief periods of time, red blood cell destruction apparently proceeded at a faster rate on a high fat diet, as evidenced by an increased urobilin excretion in the feces (Glanzmann, 1929; Salomon, 1920; Josephs, Holt, Tidwell and Kajdi, 1938). The experiments of Freeman, Loewy, Marchello, and Johnson (1942) carried out for many weeks conclusively demonstrated this effect by the more direct method of measurement of the bilirubin excretion in dogs, which was greater with increases of fat in the diet.

Some of the mechanisms involved in this blood destruction from fat ingestion were clarified by Johnson and Freeman (1938), who showed that during the absorption of a fat meal, lymph collected from the lacteals or from the thoracic duct is very hemolytic. It was also demonstrated (Freeman and Johnson, 1940) that fatty acids and soaps, which have presumably escaped resynthesis into neutral fat during absorption, are present in the lymph in quantities sufficient to account for this hemolysis.

Since the lymph entering the blood after a fat meal is hemolytic, more direct evidence of red blood cell injury in the circulating stream at that time was sought. In preliminary experiments on dogs, actual *hemolysis* did not occur when red blood cells were mixed with lipemic serum drawn after a fat meal. The experiments here reported test whether red cells are made more *fragile* by exposure to such serum.

METHODS AND RESULTS. The fragility of red cells mixed with lipemic serum was compared with that of cells similarly mixed with fasting serum. The fragility test involved identical dilutions with distilled water of both sets of blood plus serum mixtures, gentle shaking of these diluted mixtures, and finally, counting the intact cells (per cubic millimeter) in each sample. Addition of the water reduced the inorganic salt concentration to approximately the equivalent of 0.4 per cent NaCl, midway between the concentrations producing beginning and total hemolysis in dogs.

Each dog tested was fed a fat meal consisting of 10 cc. of olive or corn oil per kilogram body weight in addition to commercial canned dog food. Samples of fasting serum ("control fluid") and of lipemic serum (one of the "test fluids") from the same dog drawn at two and at three and one-half hours after a fat meal were each mixed with an equal volume of whole blood (oxalated) from the fasting

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animal. After shaking each mixture for forty-five minutes, distilled water was added and the shaking was continued for thirty minutes. The final mixture contained one volume of red cells (i.e., oxalated blood), one volume of fasting or lipemic serum, and two volumes of distilled water. Ordinary red blood cell counts were then done on each mixture.

To express the results graphically (fig. 1), the red cell count of the control mixture (e.g. fasting serum and oxalated blood) is arbitrarily placed at zero hemolysis; that is, the hemolysis from distilled water and from shaking is disregarded since this is identical in both control and test mixtures. The test

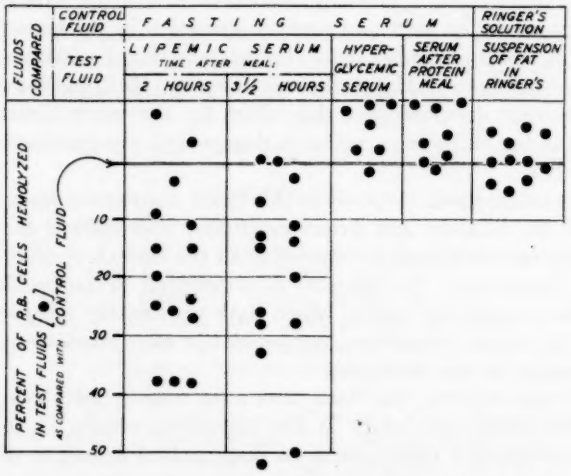

Fig. 1. Change in fragility of red blood cells upon exposure to various test fluids. The test counts are plotted as per cent reduction (the ordinate) from the corresponding control count which is represented by the zero line. To avoid confusion, these control points are omitted.

count is then expressed as percentage reduction from the control. In other words, it represents the increase in hemolysis caused by the test fluid.

When lipemic serum was the test fluid, red cell fragility was significantly increased, as indicated by the lower counts in the test mixtures analyzed statistically. There was no appreciable difference between the effects of the two and the three and one-half hour sera. The average reduction from the control for all lipemic sera tested was 19 per cent.

To check on the possibility that ingestion of other fuel foods might increase red cell fragility, high sugar and high protein meals were fed. The hyperglycemic serum and the serum after the protein meal, respectively, were used as test fluids, substituting for lipemic serum in procedures exactly parallel to those employed with high fat meals. No increase in the fragility of red cells exposed

to these sera was observed. If anything, the *resistance* of the red cells was increased in these caloric controls as indicated by the higher counts in the test mixtures (see third and fourth columns of fig. 1). However, too few experiments have been performed to warrant any final conclusion on this point. Earlier workers have reported that large quantities of sugar do exert a protective effect on red cells preserved *in vitro* (Rous and Turner, 1916). Also, insulin hypoglycemia has been shown to increase the fragility of red cells (Booth, 1941).

Although other studies (Freeman and Johnson, 1940) have shown it to be unlikely that the neutral fat particles in any way affect the fragility of red cells, it was felt worthwhile to test the possibility under the conditions of this experiment. A stable suspension of olive oil in Ringer's solution was used as the test fluid; the particle size and the concentration approximated those in lipemic serum. This was compared with Ringer's solution as the control fluid. Neutral fat was found to be as ineffective as Ringer's solution in altering red cell fragility (see last column of fig. 1). The deviation here was from −6 per cent to +5 per cent, perhaps giving a good indication of the limits of error of the method.

Discussion. These experiments demonstrate the presence in dogs' lipemic serum of one or more agents which act directly on red blood cells to increase their fragility. Probably these agents are the free fatty acids and soaps which account for the hemolytic potency of thoracic duct lymph after fat absorption (Freeman and Johnson, 1940). They apparently proceed from the lymph into the blood in sufficient concentration to cause this increase in red cell fragility. Chemical determinations of these substances in the serum after a fat meal have not been done.

The evidence strongly suggests that red cells are injured *in vivo* very soon after the products of fat digestion enter the bloodstream. It follows that the increased blood destruction during a prolonged high fat diet (Freeman, Loewy, Marchello and Johnson, 1942) may be accounted for largely or wholly by continual repetitions of this short-time effect rather than by some longer, more indirect, metabolic process. Indeed the possibility that ketone bodies may be the destructive agents has probably been eliminated in the experiments of Josephs, Holt, Tidwell and Kajdi (1938). Experiments by Freeman, Loewy and Johnson (1943) lend strong support to the concept that free fatty acids or soaps can act almost at once to increase red cell destruction. They demonstrated an increased bilirubin excretion in anesthetized dogs within an hour or two after injection of small quantities of fatty acid or soap into the jugular vein.

It may be, as perhaps suggested by the experiments where sugar and protein were fed, that the non-fat elements of the diet serve in small part to protect against the injurious effect of fat on the red blood cells. However, at least in dogs, fat ingestion probably is an important mechanism in the normal daily red cell destruction.

Considered in the light of this property of ingested fat, it seems probable that the following well-known phenomena have definite adaptive values hitherto not emphasized: 1, the tendency to vomit after a high fat meal; 2, the slower emptying time of the stomach after fat meals than after meals high in carbohydrate or protein; and 3, the absorption of fat into the lymphatic system and its subsequent gradual introduction into the circulating blood. All these prevent the digestion products of ingested fat from entering the blood stream too quickly or in too great amounts. They would appear to be part of a physiological system of checks to prevent excessive red cell destruction. The ultimate check against anemias of increased red cell destruction is the bone marrow which, in animals on prolonged fat diets, is capable of replacing the extra cell losses (Freeman, Loewy, Marchello, and Johnson, 1943). When these checks are inadequate or when the blood cells themselves are more susceptible to fat injury, an anemia may well develop. These possibilities remain to be investigated as causative factors in certain human anemias.

It is of interest that injury from fat ingestion is encountered in cells other than the erythrocytes. A number of recent animal experiments indicate that high fat diets also produce liver damage. These experiments fall into two groups: 1, those in which fat ingestion apparently damages the liver cells directly (Blumberg and McCollum, 1941; György and Goldblatt, 1941); and 2, those in which fat ingestion acts by increasing the susceptibility of the liver to damage by other agents. Deleterious agents which have been used in such experiments are chloroform (Goldschmidt, Vars, Raudin, 1939), carbon tetrachloride (Bollman, 1940) and arsphenamine (Messinger and Hawkins, 1940). In the case of arsphenamine poisoning, when high carbohydrate or protein diets were changed to high fat diets, the icterus index showed a progressive increase. The work presented here suggests that this icterus may be caused in part by increased blood destruction as well as by liver damage. If fat is injurious to both blood cells and liver cells, it would seem to increase the likelihood that fat ingestion may be related to the etiology of certain anemias.

SUMMARY AND CONCLUSIONS

1. After fat ingestion an agent which increases the fragility of red blood cells is present in dog's serum.

2. Neutral fat is not this agent. Previous work indicates that free fatty acids and soaps, reaching the blood stream by way of the lymphatics, are probably responsible for the effect.

3. Ingestion of carbohydrates and proteins does not increase red cell fragility. On the contrary, meals rich in these substances produce serum which may actually protect red cells from water hemolysis.

4. These results, when considered with the finding of increased red cell destruction *in vivo* on a high fat diet, indicate that fat ingestion probably is an important mechanism in the physiological destruction of red cells in dogs.

5. These experiments suggest further that the mechanism of the demonstrated *in vivo* red cell destruction by fat ingestion is immediate and direct and may not involve any relatively long metabolic process.

6. It is suggested that those physiological mechanisms which delay the absorption of fat and its entrance into the bloodstream are probably significant in the prevention of anemia.

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THE EFFECT OF OXYGEN DEPRIVATION ON THE RELATION BETWEEN STIMULUS INTENSITY AND THE LATENCY OF VISUAL AFTER-IMAGES

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The visual mechanism manifests marked changes in function when its oxidative processes are disturbed.[1] Such a disturbance may be caused by exposure to low atmospheric oxygen tensions, as in high altitude flying, and by reducing the supply of metabolites which can be utilized by the central nervous system, as in insulin hypoglycemia (1). During anoxia, the visual field appears to become dim. On readmission of oxygen, it appears to become brighter again. A quantitative and highly sensitive index of such changes in visual sensitivity during anoxia can be obtained by determining the absolute light threshold, as shown by McFarland and Evans (2). They found, for example, that at a simulated altitude of 15,000 feet a light intensity 2.5 times as great as normal was required in order for it to be seen.

Experimental evidence has been presented by both Crozier and Holway (3) and by Hurvich (4) in support of the view that the critical determinants of the visual sensory processes may be central rather than photochemical in nature. The effects of anoxia seem also to be attributable primarily to alterations in the *neural* elements of the visual mechanism, although the exact locus remains to be determined. The following facts indicate that the photochemical mechanism is not involved: *a.* The light sensitivity of a previously dark-adapted individual is reduced by subsequent oxygen deprivation. It is presumed that the concentration of the photosensitive substances remains unchanged, since no exposure to light has taken place. Furthermore, the decreased sensitivity could not be due to delayed regeneration of these substances, since this process had been completed before exposure to anoxia (5). *b. In vitro,* the rate of decomposition and regeneration of visual purple is not affected by the absence or presence of oxygen (6). *c.* Although different photosensitive substances are concerned with rod and with cone vision, anoxia causes an equal rise in the rod and cone thresholds (7). *d.* The threshold of the eye to electrical stimulation, which presumably does not depend on photochemical mechanisms, also rises during anoxia (8). *e.* On readmission of oxygen, the rate at which sensitivity is recovered is much more rapid than can be accounted for by the rate at which the visual pigments regenerate.

In order to determine the effect of anoxia on a visual function at illuminations of higher than threshold intensities, McFarland and Halperin (9) made a study

[1] For a comprehensive summary of the literature concerning the effects of anoxia on vision, see McFarland, R. A., J. N. Evans and M. H. Halperin. Arch. Ophthal. **26:** 886, 1941.

of foveal visual acuity in relation to illumination. In normal air, the logarithm of visual acuity rises continuously as the logarithm of illumination intensity is increased—rapidly at first, then more gradually until a maximum is reached and the curve becomes horizontal. During exposure to low oxygen tensions, the form of this curve remains unaltered, but the curve as a whole is translated to the right along the intensity axis. The amount of this translation is of the same order of magnitude, in logarithmic units, as in the case of the absolute thresholds at comparable degrees of anoxia. Studies of other visual functions, such as intensity discrimination (differential sensitivity), have yielded similar results (10).

The relationship between oxygen deprivation and the latency of visual after-images has been investigated in two previous studies. Gellhorn and Spiesman (11) performed experiments on subjects who breathed gas mixtures deficient in oxygen at sea-level pressure. The stimuli were colored squares or a white light, and the fixation time ten seconds. Whereas a reduction of the oxygen concentration to 13 per cent was without effect on the latency of the after-image, changes of considerable magnitude occurred when air containing 9 to 11 per cent oxygen was breathed. Under these conditions, the after-images were either delayed, or they failed to appear at all. In subjects acclimatized to high altitudes during the International High Altitude Expedition to the Chilean Andes, McFarland (12), using a similar method, likewise found a lengthening of the latent period. This prolongation became statistically significant only at 21,100 feet, which corresponds to an oxygen concentration of 9.5 per cent at sea-level pressure.

Certain experimental limitations of these earlier studies, as, for example, their restriction to a single value of the stimulus intensity continuum, suggested the advisability of a more extensive investigation of the behavior of after-images during anoxia. In the present study the intensity of the stimulus was varied over a wide range so that the behavior during anoxia of the entire function might be observed. The present experiments differ from the earlier ones also in employing a brief stimulus duration of 0.2 second. The relationship between stimulus intensity and the latency of after-images has been studied under a wide variety of experimental conditions in normal air (13–17). The results differ widely and depend on such variables as the state of retinal adaptation and the exposure time, size, the retinal location, and spectral composition of the stimulus, as well as the background against which the after-image is observed. The problem becomes complicated when prolonged stimulus exposures are employed, since the eye cannot be maintained motionless for more than a fraction of a second, and adjacent retinal areas are unequally exposed to the stimulus when the eye moves. Also, observation of the after-image against an externally illuminated background results in the appearance of "positive" and "negative" phases of the after-image. Such complicating factors can be minimized by utilizing stimuli of very short duration and observing the after-image in complete darkness. In this manner, fixation is more easily controlled and only "positive" after-images (i.e., images brighter than the background) are seen.

Even under such simplified conditions, the results obtained seem to depend on the intensity range. Hurvich and Joensson (17) made a systematic investigation of the latent time of a bluish-gray after-image which appears approximately 0.5 to 1.5 seconds after cessation of a brief white stimulus. The latent time of this image *decreased* with an increase of stimulus intensity, duration, or area. When the magnitude of any one of these variables was raised beyond certain limits, there was a brilliant after-glow following the cessation of the stimulus, which obscured the appearance of the usual bluish-gray after-image. Feinbloom (16) also used brief stimuli and observed the after-image in complete darkness. He employed, however, a range of stimulus intensities beginning at a higher brightness level than did Hurvich and Joensson. The positive after-image which he observed appeared several seconds after cessation of the stimulus. The latent time of this image *increased* with an increase of stimulus intensity, duration, or area. The after-images described by Hurvich and Joensson differed also in certain qualitative characteristics from those reported by Feinbloom. Whereas the former found the color of the after-image to be bluish-gray regardless of the spectral composition of the stimulus, the latter reported the color of the after-image to vary with the color and intensity of the stimulus.

The present study was performed with the same apparatus and under the same experimental conditions as employed by Hurvich and Joensson (17). The after-image observed under these conditions is described by them as corresponding to that known traditionally as the tertiary or Hess image.

Apparatus. The various components of the optical system employed were mounted on an optical bench and arranged so as to provide independent control of three stimulus variables: (1) intensity, (2) duration and (3) area. In this study only the first of these was varied.

The light source was a 100-watt, 115-volt Mazda projection lamp. A lens served to condense the light rays upon the surface of a milk glass plate (3 x 3 cm.). The latter was placed so that the aperture of the stop delimiting the size of the stimulus field was uniformly illuminated. An aperture of 11.9 mm. diameter, subtending an angle of two degrees at the eye of the observer, was employed. Intensity was controlled by the use of neutral-tint Wratten filters and a specially mounted pair of optical wedges arranged in compensating fashion. The duration of the stimulus exposure was kept constant throughout these experiments at 0.2 second by means of a Compur camera shutter placed between the wedges and an artificial pupil. The latter was 1 mm. in diameter. A synchronizing switch mounted on the shutter made it possible to start an electric timing clock automatically at the end of the stimulation period. The observer's eye was placed at a distance of 35 cm. from the plane of the aperture. A Bausch and Lomb head rest and chin support facilitated the maintenance of fixation. A red fixation dot, 1 mm. in diameter, was continuously visible, being reflected from a glass wafer set at an angle of 45 degrees to the line of regard. The fixation dot was centered in the stimulus field and was also at a distance of 35 cm. from the observer. Extraneous light stimulation was eliminated by placing the observer in a light-proof cubicle painted black.

For the adapting field, a large white matte surface subtending 50 x 65 degrees was fixed on the wall of the chamber adjacent to the apparatus at a distance of 50 cm. from the observer's eye. The intensity of light reflected by this adapting field was kept constant throughout the experiments at approximately ten foot candles.

The measured time interval—i.e., the time elapsing between the cessation of the stimulus and the signal of the occurrence of the after-image—is termed the latent period. To measure this interval, a six-volt battery was connected in series with a precision-timer[2] reading in hundredths of a second, the synchronizing switch on the camera shutter, and a signal key manipulated by the observer. Before each stimulus presentation, the observer depressed the signal key. At the moment the stimulus field was cut off, the shutter switch was automatically tripped. This completed the circuit and thus started the timer. The latter stopped when the observer signalled the onset of the after-image by breaking the circuit as he released the depressed key.

EXPERIMENTAL PROCEDURE. The experiments were performed in a sealed chamber which permitted the regulation of temperature and ventilation by means of an air-conditioning unit, and the variation and maintenance of the oxygen content of the air at any desired level, the total barometric pressure remaining constant.

The data required for the determination of each curve relating stimulus intensity to latency of the after-image were obtained as follows. Observations were preceded by three minutes of bright adaptation to the ten foot-candle adapting field, followed by five minutes of dark adaptation. All observations were made with the right eye. The measurements were taken in such a sequence as to be homogeneous for intensity. A single measurement was made consecutively at each intensity, passing usually from the lowest to the highest. The direction was then reversed until either three or five readings had been obtained at each point. Following each single determination the observer was given a thirty-second rest period. Additional pauses were introduced at the end of each series (one reading at each of the four or five intensities), and whenever the observer reported fatigue or persistent visual after-effects.

The procedure thus outlined was first carried out in normal air (21 per cent oxygen). Samples of the subject's normal alveolar air were obtained. The concentration of oxygen in the chamber was then reduced to the desired level in about twenty minutes by diluting the air with nitrogen. After about ten minutes' exposure to the low oxygen tension, the experimental procedure was repeated. Samples of the chamber air and of the subject's alveolar air were obtained before and after the visual measurements. They were subsequently analyzed for oxygen and carbon dioxide on a standard Haldane apparatus. The determinations at the simulated high altitude were followed by administration for stated intervals, of 100 per cent oxygen through a mask. The after-image measurements were then repeated ("recovery curve").

[2] Model S-1, Standard Electric Time Company, Springfield, Mass.

Means and standard deviations (S.D.) were computed for the three or five observations at each stimulus intensity by the usual methods. In those instances in which combined means are given for several experiments (table 1), the combined standard deviation at each intensity was computed according to the equation S. D. $= \sqrt{\frac{\Sigma p\sigma}{n-r}}$, where p is the number of individual observations

TABLE 1

The effect of oxygen deprivation on the latency of visual after-images

(See fig. 1A, B, and C)

SUBJECT	LOG_{10} STIMULUS INTENSITY (PHOTONS)	LATENT TIME OF AFTER-IMAGES (SECONDS)*					
		Normal air		Low oxygen†		Recovery with oxygen‡	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
LMH (mean of 3 experiments§ at 10.4–11.2 per cent oxygen)	1.16	1.33	0.08	1.51 (11)*	0.13	1.27	0.20
	1.48	1.17	0.06	1.33	0.13	1.15	0.19
	2.13	1.08	0.07	1.14	0.10	1.10	0.10
	2.79	0.93	0.06	1.02	0.08	0.96	0.06
JDM (mean of 2 experiments at 11.2–11.3 per cent oxygen)	1.20	1.33	0.07	1.53 (3)	0.11	1.35	0.14
	1.52	1.16	0.11	1.30	0.12	1.16	0.15
	2.17	0.87	0.12	0.97	0.21	0.83	0.05
	2.82	0.59	0.07	0.75	0.13	0.67	0.12
MHH (one experiment at 12.5 per cent oxygen)	1.16	1.62	0.37	1.77	0.30	1.60	0.23
	1.48	1.11	0.14	1.45	0.28	1.04	0.26
	2.13	0.85	0.06	0.90	0.03	0.88	0.04
	2.79	0.82	0.10	0.77	0.04	0.72	0.12

* The number of observations represented by each datum is as follows: subject LMH, 13; subject JDM, 8; subject MHH, 5. Numbers in parentheses indicate the number of measurements on which the mean is based in those cases in which the after-image failed to appear during one or more observations.

† See table 4 for the individual alveolar air and chamber air analyses.

‡ The time during which oxygen was breathed subsequent to exposure to the reduced oxygen tension ("recovery time") was as follows: subject LMH, 45–50 minutes; subject JDM, 25–38 minutes; subject MHH, 17 minutes.

§ Recovery curves were obtained in only two of these three experiments. In order to make the mean of these two comparable to the mean of all 3 experiments in normal air, a correction factor was applied. This was derived from the ratio of the means for all three normal-air curves to the means for the normal-air curves in these two experiments.

and σ the standard deviation of these observations in each experiment, n the total number of observations and r the number of experiments.

RESULTS. The relationship between the latency of visual after-images and stimulus intensity,[3] during exposure to various atmospheric oxygen tensions, is

[3] Stimulus intensity is expressed in photons (millilamberts $\times \frac{10}{\pi} \times$ pupil area in square millimeters).

shown in table 1 and figure 1 (A, B and C). These data are based on six experimental sessions employing three trained subjects. Each session consisted of a series of measurements in normal air (21 per cent oxygen), followed by repetition of the determinations during anoxia. Finally, 100 per cent oxygen was administered and observations were repeated after stated recovery intervals.

The general form of the function relating latent time to stimulus intensity is curvilinear. The latent time decreases as the stimulus intensity is increased—rapidly at first, then more gradually. Furthermore, a given degree of anoxia causes a delay in the appearance of the after-image over the entire range of stimulus intensities, the magnitude of the delay being a function of the stimulus intensity.

The limited accuracy of the measurements precludes the possibility of a unique mathematical description of the displacement of the curves caused by anoxia—

TABLE 2

The effect of progressive degrees of anoxia on visual after-images

Data from one experiment on subject MHH (see fig. 1D)

$\log_{10}$ STIMULUS INTENSITY (PHOTONS)	LATENT TIME OF AFTER-IMAGE (SECONDS)*									
	Normal air		13.7% oxygen		12.1% oxygen		10.8% oxygen		Recovery with oxygen†	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
0.60	1.38	0.09	2.06	0.44	1.86	0.08	2.19	0.34	1.14	0.05
0.93	1.13	0.14	1.45	0.18	1.53	0.09	1.78	0.04	1.01	0.06
1.56	0.98	0.11	1.11	0.09	1.29	0.21	1.58	0.19	0.91	0.02
2.20	0.78	0.06	0.87	0.06	0.82	0.07	1.10	0.17	0.70	0.04
2.84	0.65	0.06	0.66	0.05	0.72	0.08	0.96‡	0.06	0.68	0.08

* Each entry is the mean of three observations.

† After twenty minutes.

‡ Mean of two observations. After-image failed to appear once.

i.e., whether it is horizontal, vertical, or both. Relatively more precise measurements of visual acuity (9) and intensity discrimination (10) have shown that during anoxia the curves relating these variables to the logarithm of stimulus intensity are displaced solely on the intensity axis. The curves in figure 1 were, therefore, drawn in such a manner as to determine whether the effect of anoxia on the latent time might similarly be described in terms of a horizontal translation along the intensity axis. A curve was first fitted by visual inspection to one set of points in each graph. The *same curve* was then translated horizontally until it fell on the other set, extrapolating when necessary. In each case, the curve which fits the normal air data was found to fit those for low oxygen as well, when transposed on the intensity axis. The form of the curve is thus identical under all the conditions tested. The effect of anoxia may be described simply as a translation of the curve to the right along the intensity axis, causing a prolongation of the latent time for any given stimulus intensity.

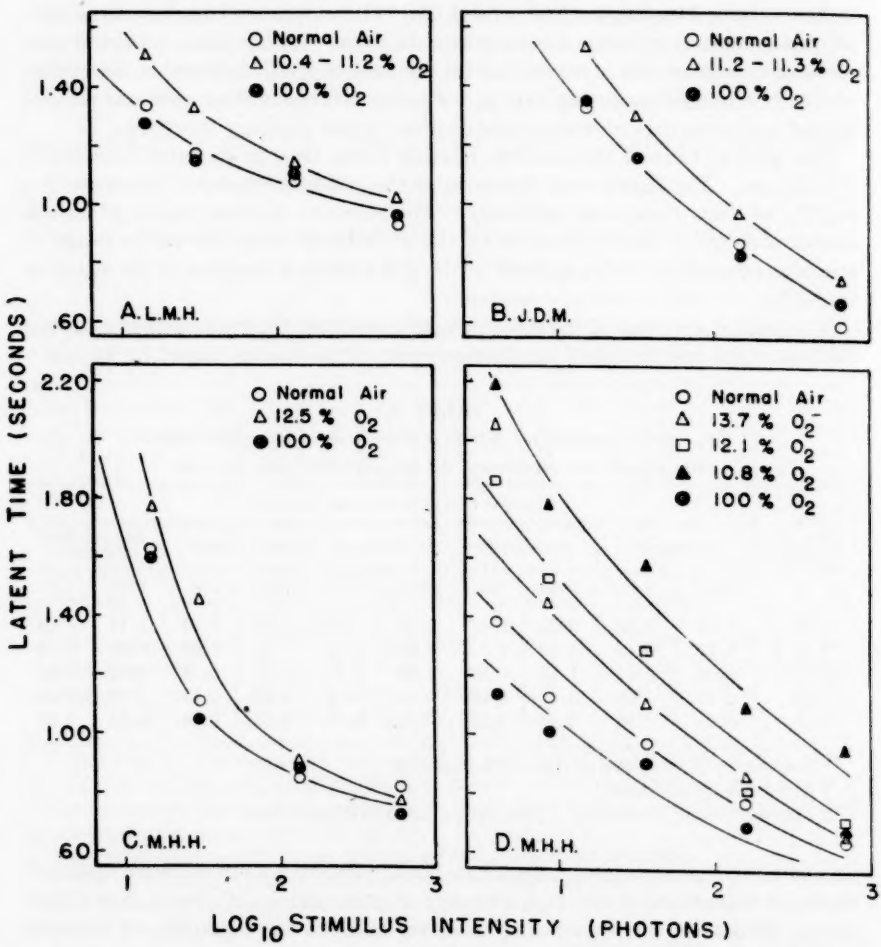

Fig. 1. The effect of anoxia on the relation between the latent time of visual after-images and stimulus intensity. Graphs A, B and C are based on the data in table 1, and graph D on those in table 2. In each case, the observations were made under the conditions and in the sequence indicated by the corresponding legend.

A curve was first fitted to one set of points in each graph and then translated horizontally until it fell on the other set or sets of points extrapolating when necessary. (In graphs A, B and C, the open and solid circles were treated as a single set.) Since in each graph a satisfactory fit to each set of points could be obtained by horizontal translation of the same curve, the effect of anoxia may be described as a displacement of the curve to the right on the intensity axis. This results in a prolongation of the latent time for any given stimulus intensity.

This translation is similar in direction and magnitude (when expressed in logarithmic units of intensity) to that observed in the case of foveal visual acuity (9) and intensity discrimination thresholds (10), during comparable degrees of anoxia. The meaning of such a translation in each of the three cases is that a greater intensity of light is necessary during anoxia to produce a given visual response. This is equivalent to the effect of a reduction in the intensity of the stimulus by placing a light filter or smoked glass before the observer's eye. At low intensities of illumination, where the curves are steepest and a small decrease in illumination causes a large change in the visual function, anoxia similarly causes a large change. At progressively higher intensities of illumination, where the curves are less steep and a proportionate decrease in illumination causes less change in the visual function, anoxia likewise has a progressively smaller effect. This dependency of the magnitude of change in latent time during anoxia on stimulus intensity is confirmed by the data in table 1. These indicate that the prolongation of latent time is greater for stimuli of low intensity than for more intense ones. In fact, the least intense stimulus employed, which always yielded an after-image in normal air, frequently failed to produce one during oxygen deprivation, the latency thus being prolonged indefinitely. For example, in the case of subject JDM, during anoxia an after-image appeared in only three of a total of eight trials at the lowest intensity.

The effect of successive exposure to three progressively more severe degrees of oxygen lack is shown in figure 1 (D) and table 2, which present the data for one subject. A curve was fitted to one set of points by visual inspection and translated horizontally to fit the other sets. In general, the extent to which the curve is displaced varies directly with the degree of oxygen deprivation. There is, however, some overlapping of the data at the first two stages of oxygen lack. This experiment, in which only three observations were made at each point, required approximately five hours. Because of the small number of observations which were thus possible, conclusions as to the quantitative relation between the degree of oxygen deprivation and the change in the latency of the after-images cannot be drawn. Furthermore, control experiments on other subjects, who made an equal number of observations over a period of four hours in normal air, also showed a tendency toward a lengthening of the latent time toward the end of the experiment, possibly as a result of fatigue. It is possible, therefore, that a part of the change at the lowest oxygen concentration may also be attributable to fatigue. The complete reversal of the change upon administration of oxygen suggests, however, that the prolongation was due primarily to anoxia. Here, again, the changes were greatest at the lowest intensities (where the curve is steepest) and least at the brightest intensities (where the curve is less steep).

Experiments on light sensitivity (2) and on foveal visual acuity (9) have indicated that when oxygen is administered subsequent to oxygen deprivation full recovery of these functions takes place in three or four minutes. It was anticipated, therefore, that the latent time of the after-images would behave in a similar fashion. Preliminary experimentation indicated very clearly, however, that three or four minutes of oxygen inhalation failed to produce even an appre-

ciable degree of recovery. As table 3 and figure 2 indicate, the curve relating latency to stimulus intensity does not return to its original position even after a

TABLE 3

Slow recovery of latent time of after-image subsequent to anoxia

Data from one experiment on each of two subjects (see fig. 2)

SUBJECT	LOG_{10} STIMULUS INTENSITY (PHOTONS)	LATENT TIME OF AFTER-IMAGE (SECONDS)*							
		Normal air		10.4% oxygen		Recovery with oxygen—10 min.		Recovery with oxygen —50 min.	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
MHH	0.85	1.97	0.22	2.34	0.35	1.99	0.21	1.91	0.20
	1.16	1.38	0.30	1.80	0.09	1.50	0.21	1.37	0.16
	1.48	1.10	0.08	1.42	0.15	1.31	0.19	1.12	0.15
	2.13	0.89	0.10	0.94	0.09	0.91	0.10	0.87	0.09
	2.79	0.69	0.13	0.89	0.16	0.75	0.06	0.63	0.07
LMH	1.16	1.31	0.07	1.65	0.16	1.41	0.13	1.32	0.13
	1.48	1.25	0.06	1.38	0.08	1.37	0.12	1.21	0.09
	2.13	1.06	0.06	1.16	0.08	1.17	0.06	1.16	0.09
	2.79	0.97	0.03	1.10	0.09	1.02	0.06	0.96	0.04

* Each entry is the mean of 5 observations.

TABLE 4

The mean oxygen and carbon dioxide content of the alveolar air and of the chamber air during the experiments reported in tables 1, 2 and 3

	SUBJECT	ALVEOLAR AIR				CHAMBER AIR		
		Normal		In low oxygen		CO_2	O_2	Equivalent altitude
		CO_2	O_2	CO_2	O_2			
		mm. Hg	*mm.* Hg	*mm.* Hg	*mm.* Hg	*per cent*	*per cent*	*feet*
Table 1	LMH*	38	100	32	40	0.22	10.81	17,000
	JDM†	38	103	35	41	0.29	11.24	16,000
	MHH	39	99	34	50	0.20	12.52	13,300
Table 2	MHH‡	39	99					
	1st alt.			37	57	0.21	13.66	11,000
	2nd alt.			36	46	0.23	12.14	14,000
	3rd alt.			32	31	0.50	10.80	17,000
Table 3	MHH	42	95	34	35	0.18	10.51	17,600
	LMH	38	97	32	35	0.18	10.36	18,000

* Mean of 3 experiments.

† Mean of 2 experiments.

‡ Three successive "altitudes" in single experimental session.

ten-minute recovery period. It should also be noted that oxygen was still being breathed during these observations which lasted twenty minutes. After oxygen had been breathed for fifty minutes, the measurements were repeated and com-

plete recovery was found to have taken place. Apparently, the severity of anoxia is a factor in determining the recovery time. In an experiment on subject MHH (table 1, fig. 1 (C)) a seventeen-minute recovery period sufficed after exposure to 12.5 per cent oxygen whereas a longer period was necessary after exposure to 10.5 per cent oxygen (fig. 2 (A)). Similar findings in relation to

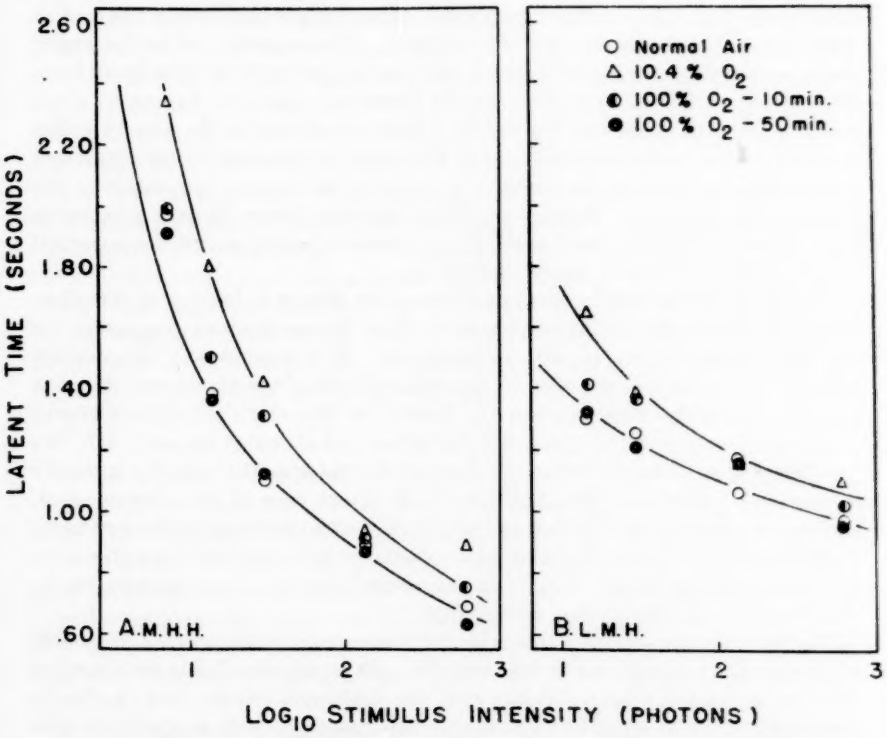

Fig. 2. The slow recovery of the latent time subsequent to anoxia. The half-solid circles, representing measurements begun after oxygen had been breathed for ten minutes, indicate that only partial recovery had taken place. After fifty minutes with oxygen, recovery was complete. This may be contrasted with the rapid recovery of light sensitivity, which is complete within several minutes when oxygen is administered after oxygen-deprivation.

The two curves in each graph are identical, a curve having been fitted to one set of points and translated horizontally to fall on the other set. The open and solid circles were treated as a single set.

recovery from anoxia have been reported by Gellhorn and Spiesman (11), who observed that in some cases the latent period remained lengthened ten minutes after exposure to atmospheres deficient in oxygen.

The results of the analyses of the alveolar air and chamber air samples obtained during the experiments described in tables 1, 2, and 3 are presented in table 4. The findings are consistent with those of earlier experiments.

DISCUSSION. The measure of latent time employed in these experiments is of necessity a composite one, involving not only the true latent time of the visual function, but also the reaction time of the observer. The question arises, therefore, as to how much of the observed change during oxygen deprivation is due to each of these components. Earlier experiments on simple reaction time (18) have shown a prolongation of only about 0.02 second under conditions of anoxia even more severe than those in this experiment. The changes observed in the present study are of a much greater order of magnitude. Consequently, by far the largest proportion of the observed difference is due to an alteration in the true latent time. Furthermore, the fact that after-images frequently failed to appear at all in response to weak stimuli during anoxia indicates a change in the sensory rather than the motor mechanism involved in the subject's response. Prolongation of the reaction time would be expected to result in an upward translation of the curve on the time axis. It is not possible to ascertain from the data obtained in this experiment whether the translation of the curve during anoxia has a vertical component in addition to the horizontal one.

An analysis of the mechanisms underlying the change in latency of the after-image during anoxia cannot be presented, since the mechanisms responsible for the after-images in normal air are unknown. In a general way the relation between the latency of the after-image studied in this experiment and stimulus intensity resembles that between the latency of the electrical after-discharge observed in studies of the optic nerve potential and stimulus intensity (17, 19). The latent time of each of these phenomena decreases as the stimulus intensity is increased. The order of magnitude of the latent time of the after-image is, however, much greater than that of the electrical after-discharge in the organisms studied to date. No reports have been published which deal with the optic nerve potentials during oxygen lack. Further correlation of these phenomena is, therefore, not possible at the present time.

The fact that the action of anoxia on the latency of after-images is comparable to decreasing the brightness of the stimulus (the latent time being prolonged in both cases) suggests the possibility that the prolonged latency may be closely associated with the apparent dimming of the stimulus which is known to take place during oxygen deprivation. However, when oxygen is administered after exposure to low oxygen tensions, the visual field rapidly becomes brighter; light thresholds return to normal levels within three to four minutes. Yet the latency shows practically no recovery after one breathes oxygen for a similar length of time. Consequently, other factors than those causing the change in apparent brightness of the stimulus must be involved and are probably related to a change in the velocity of the physiological processes underlying the eventuation of the after-image. Additional evidence that the changes in some visual functions during anoxia are not due solely to the factors producing the change in apparent brightness of the stimulus has been brought forward by Berger, McFarland, Halperin and Niven (20). They studied the effect of oxygen lack on the resolving power of the eye, employing as a test object two luminous points on a dark ground. The resolving power, as measured by this method and in the intensity

range employed, improves when the brightness of the test points is decreased. It was expected, therefore, that anoxia, which is associated with an apparent dimming of the test points, would also improve the resolving power. On the contrary, an impairment took place, indicating the operation of other factors.

The practical importance of after-images in relation to anoxia is probably not as great as that of visual acuity or light-sensitivity. The slowness of the recovery of this function when oxygen is breathed after exposure to low oxygen tensions may, however, be of practical significance. The behavior of the visual mechanism is believed to reflect changes in the central nervous system, since the retina is embryologically a part of the central nervous system and resembles it metabolically and anatomically. The slowness of the recovery of the function studied here may, therefore, be indicative of a similar delay in the recovery of other central nervous functions. This emphasizes the importance of the use of oxygen by airplane personnel at high altitudes as a preventive measure before any appreciable effects of anoxia occur. If, instead, oxygen is used only after the changes have already taken place, a comparatively long time may be required to reverse them. The possibility that some central nervous functions recover slowly after anoxia suggests, furthermore, that certain factors contributing to landing accidents at sea-level after high altitude flights without an adequate oxygen supply may be attributable to the residual effects of oxygen lack.

SUMMARY

1. The effect of oxygen deficiency on the latent time of the "tertiary" visual after-image was measured in a low oxygen chamber over a wide range of stimulus intensities. Nine experiments were performed on three trained observers.

2. In normal air, there is an inverse relationship between the latent time of the after-image and the intensity of the stimulus; a reduction of the intensity of the stimulus prolongs the latency of the after-image.

3. Anoxia prolongs the latency of the after-image. This effect is consistent with the apparent dimming of the visual field which occurs during oxygen deprivation.

4. The magnitude of the increase in the latent time during anoxia is inversely related to the intensity of the stimulus.

5. During oxygen deprivation, the curve relating the latent time of the after-image to the logarithm of stimulus intensity is translated horizontally to the right on the intensity axis. This displacement is in the same direction and of the same order of magnitude as in the case of the curves relating foveal visual acuity and intensity discrimination to the logarithm of stimulus intensity. The data are not sufficiently precise to rule out the possibility of a slight vertical translation as well.

6. The complete recovery of the latent time upon administration of oxygen may require up to fifty minutes, depending on the degree and duration of the antecedent oxygen deprivation and other factors. The apparent brightness of the visual field, and light thresholds, is restored to normal within three to four minutes. Consequently, the factors underlying the apparent dimming of the

stimuli cannot be the only ones responsible for the prolongation of the latent time of the after-image.

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THE EFFECT OF CYANIDE AND OTHER METAL BINDING SUBSTANCES ON THE PHARMACOLOGICAL ACTION OF EPINEPHRINE[1]

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In the course of studies on the mechanism of secretion of the intraocular fluid, it has been shown that the secretory activity of the ciliary body is intimately connected with the existence in the tissue of a chain of redox reactions which link the cytochrome oxidase systems of the epithelium with the dehydrogenase enzymes and substrates of the stroma (1). This redox chain can be activated by the presence of minute amounts of epinephrine in the tissue (2). These experiments have not revealed whether some derivative of epinephrine enters directly as a mediating link in the redox chain, or whether the activation occurs in some more indirect manner. However, the first of these alternatives is at least possible for it has been shown by direct enzymatic studies that a derivative of the oxidation of epinephrine by the cytochrome system or by polyphenol oxidase can act as an oxidative mediator (3).

In view of these findings it was natural to inquire whether the pharmacological activity of epinephrine[2] on other adrenergic organs might occur through the activation by epinephrine of some redox pathways in the tissue metabolism which are not utilized by the tissue in its resting state. This large question regarding the mechanism of pharmacological action of epinephrine, we have not so far been able to subject to direct experimental test. A preliminary approach to the problem, however, is furnished by the following argument. The oxidative chains which we are considering require as their first step the oxidation of epinephrine by an iron or copper containing system. Such a system would be expected to be cyanide sensitive. The degree of cyanide sensitivity of the adrenergic reaction of various smooth muscle preparations is readily susceptible to experimental investigation. The present report concerns experiments undertaken with this end in view.

The muscle of the rabbit's small intestine is a particularly favorable organ for these experiments since, when suspended in glucose-Tyrode solution, this tissue undergoes continuous rhythmic contractions. The administration of epinephrine causes an arrest of these contractions. On such an organ it is readily possible, therefore, to distinguish the effect of a small dose of cyanide which inhibits the epinephrine effect from that of a larger dose of cyanide which abolishes the rhythmic contraction of the muscle altogether. Most of our experiments were, therefore, performed on this organ. It is possible, however, to extend these studies also to organs in which epinephrine excites muscular contractions.

[1] This work was supported in part by the John and Mary Markle Foundation.

[2] The epinephrine for our experiments was kindly supplied to us by Parke, Davis & Co.

When, in such an organ, the response to epinephrine has been abolished by cyanide, it is still possible to distinguish between an inhibition of the adrenergic response and a paralysis of the muscle by testing the response to some other pharmacodynamic agent. Experiments of this type will also be reported below.

Scattered and somewhat conflicting reports are to be found of experiments similar to those here contemplated. Evans (4) found that the blood pressure response to epinephrine was depressed in cyanide poisoning though the vessels still responded to pituitrin and barium. However, he reports synergism between cyanide and epinephrine on the intestine. Masing (5) found that cyanide inhibited contraction of the hepatic vessels by epinephrine but found that the glycogenolytic effect of epinephrine was not inhibited by cyanide. This latter is in agreement with Cori's conclusions that the glycogenolytic effect of epinephrine involves essentially anaerobic mechanisms (6). Hazama (7) reported that cyanide in small doses abolishes the inhibitory effect of epinephrine on the intestine and itself caused slight excitation of this organ and Starkenstein (8) found that cyanide excited contractions in the epinephrine inhibited intestine.

Our own experiments were performed exclusively on excised organs suspended in Tyrode solution, thus avoiding the possibility of complex and indirect effects that may be present in experiments on the intact animal. However, experiments on such isolated tissues are also not devoid of their own special complications. In particular we found it necessary early in our work to attempt to distinguish between effects taking place in the bathing fluid and those taking place within the tissue. It is well known that epinephrine undergoes oxidation when placed in Tyrode solution and rapidly loses its pharmacological potency. The suppression of heavy metal catalysis of such oxidation by cyanide would lead to a larger effective dose of epinephrine reaching the tissue when cyanide is present in the bathing fluid than when cyanide is absent. In order to avoid the confusion of true pharmacological effects by chemical reactions occurring in the bathing fluid it was, therefore, necessary for us to find some way of controlling the oxidation of epinephrine in the bathing fluid.

Bathing fluid effects. The literature on "auto-oxidation" of epinephrine is quite extensive and has recently been reviewed by Starkenstein (8), and by Clark and Raventós (9). A great variety of substances have been found to inhibit the auto-oxidation of epinephrine in vitro, and several of these have been shown to enhance and prolong the response to epinephrine of various tissue preparations. Among the factors which enhance the rate of epinephrine auto-oxidation are: high pH, traces of iron or copper, exposure to glass surfaces. In Tyrode solution, the rate of auto-oxidation of epinephrine is 5 times that in blood.

Among the substances which diminish the auto-oxidation of epinephrine are pyrogallol, ascorbic acid, glutathione, amino acids, serum and tissue extracts. It is likely that what has been called "sensitization to epinephrine" by serum in the pharmacological assay technique (Schlossmann and others) (10) is due to prevention of auto-oxidation. The same holds true for sodium citrate which is recommended as a sensitizer in epinephrine assay by Stuber (11).

Our experiments were performed on isolated rabbit's small intestine and on non-pregnant rabbit's uterus using the arrangement of Magnus (12).

The test tissue was suspended in 100 cc. well aerated Tyrode solution containing 0.1 per cent dextrose. The ratio of bathing fluid volume to tissue volume is important in these experiments. If the fluid volume is greatly reduced, for instance to 10 cc. for 1 gram of tissue, the bathing fluid effects reported below are much less striking. The temperature was kept between 38 and 40°C. by a water bath. The pH of the bathing fluid was approximately 8.5. The animals from which the tissue was taken were either anesthetized with nembutal or killed by intravenous air injection or by a blow on the neck. The intestine preparations (usually ileum) were used on the day of their removal. Uterus preparations were used either fresh or after storage in the refrigerator for a day or two.

In the search for a satisfactory method of controlling auto-oxidation of epinephrine in the bathing fluid, various substances were added to the Tyrode solution immediately before the addition of epinephrine. In table 1 are listed some substances which may variously be thought of as antioxidants or as metal binders which we have found to enhance the reactivity of the tissue to epinephrine. All these substances have the same effect (fig. 1). In the dosage used,

Fig. 1. Effect of the presence of 8-hydroxyquinoline (HQ) in the bathing fluid on the response to epinephrine (Adr.). Rabbit's intestine.

none of them caused any pharmacodynamic reaction of the muscle preparation, but the reaction produced by epinephrine added to the Tyrode solution shortly after any of these substances was much more intense and much more prolonged than that produced by the same dose of epinephrine when these substances were absent.

Attention is particularly directed toward those colloids—lens proteins, egg albumin, gelatin, gum arabic—which when added to the Tyrode solution produced the same effect as the antioxidants and metal binders. These colloids were thoroughly dialyzed against distilled water before use in these experiments and their effects constitute conclusive evidence that the phenomenon observed concerns reactions in the bathing fluid, not in the tissue. The protecting substances must be added to the Tyrode solution at least a few seconds before the epinephrine. If added simultaneously with the epinephrine, even if mixed with the epinephrine before addition, their effect is small or absent. It follows that the primary reaction of these protecting agents is with some substance in the Tyrode solution (probably copper) not with epinephrine. Lens protein and egg albumin cause the Tyrode solution to foam badly and were, therefore, not used

in subsequent experiments. Gelatin, if allowed to stand for a long time in well aerated Tyrode solution, finally loses its protective value—possibly through oxidation of its sulfhydryl groups. In the experiments to be reported below, gelatin was added to the bathing fluid only shortly before epinephrine. Gum arabic, on the other hand, keeps its protective capacity for a long time and may be added in the stock Tyrode solution.

Effects of cyanide. The effect of cyanide on the epinephrine response was first tested on the rabbit's intestine. In order to distinguish between reactions of cyanide in the bathing fluid and genuine pharmacological effects, the cyanide experiments were performed both with and without the addition of gelatin or gum arabic to the bathing fluid. In general, the cyanide was added one-half to one minute after the gelatin or gum arabic and the same interval before the epinephrine. If no gelatin or gum arabic is used, three different effects of cyanide can be observed. In very low concentration[3] (about 2.10^{-6} molar) cyanide abolishes or diminishes the effect of a small dose of epinephrine given subse-

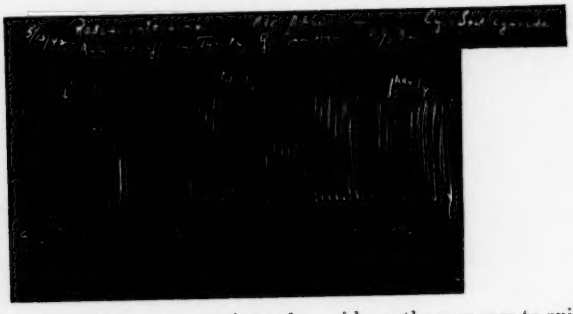

Fig. 2. Effect of medium concentrations of cyanide on the response to epinephrine in the presence of gum arabic in the bathing fluid. Intestine.

quently. In somewhat higher concentration (about 10^{-5} molar) cyanide enhances and prolongs the effect of epinephrine. In still higher concentrations (about 6.10^{-5} molar) cyanide abolishes the normal peristalsis of the tissues. The effect of the medium concentrations of cyanide resembles that of protective antioxidants or metal binders in the bathing fluid. That this is a correct interpretation is shown by experiments with gelatin or gum arabic in the bathing fluid. With these protecting substances present, only two effects of cyanide can be distinguished. With low and medium doses of cyanide, the reaction to epinephrine is diminished or inhibited (fig. 2). With high doses of cyanide, the muscle is paralyzed.

The inhibition of the adrenergic response produced by cyanide is complete only for very small doses of epinephrine. If larger doses of epinephrine are used, the inhibition of cyanide is overcome. The inhibition may be competitive but quantitative relations could not be worked out since it was impossible to use large

[3] The concentration levels at which these effects were obtained varied considerably in different tissue preparations.

doses of cyanide to balance large doses of epinephrine on account of the resulting paralysis of the muscle. The antagonism between epinephrine and cyanide can be exhibited also if cyanide is added after the epinephrine, in which case the adrenergic response is rapidly terminated.

Precisely similar results were obtained on the uterus preparation (fig. 3) except that, since the response of this organ to epinephrine consists in a contraction of the muscle, the suppression of the adrenergic response by small doses of cyanide could not be immediately distinguished from a paralysis of the muscle produced by high doses of cyanide. This distinction could, however, be made by stimulating the muscle either with pituitrin or with larger doses of epinephrine.

Other substances which have a cyanide-like effect. In order to throw light on the mode of action of cyanide in inhibiting adrenergic reactions, we have sought for other substances which might have a similar effect.

Several of the substances listed by Stotz, Harrer and King (13) and by Graubard (14) as copper binders were tested. A number of these gave excellent bath-

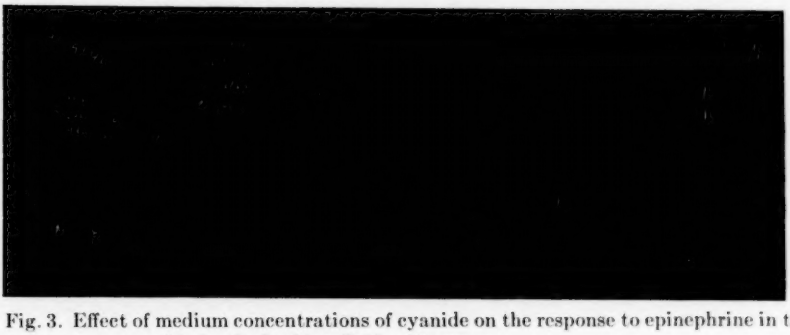

Fig. 3. Effect of medium concentrations of cyanide on the response to epinephrine in the presence of gum arabic in the bathing fluid. Uterus.

ing fluid protection and are listed in table 1. The results with these substances—xanthate, dithiocarbamate, hydroxy-quinoline—suggest that the epinephrine destroying catalyst in the bathing fluid may be copper. Two substances among the "copper binders" showed effects similar to cyanide. These were thiourea and allyl thiourea in concentrations of about 10^{-3} molar and 10^{-4} to 10^{-3} molar rsp. In the presence of gelatin or gum arabic these substances inhibit or diminish the adrenergic response both of the intestine and of the uterus. As in the case of cyanide, the inhibition could be overcome by larger doses of epinephrine. The toxic effects of larger doses of the thioureas were not investigated. In the absence of gelatin or gum arabic, threshold doses of the thioureas inhibited the adrenergic reaction. Larger doses yielded enhancement of the adrenergic reaction attributable to a bathing fluid effect. In this respect also these substances closely resemble cyanide.

Discussion. The biochemical effects of cyanide have generally been classed as falling into three types of primary reactions: metal binding, ketone binding, reduction. Thiourea can bind heavy metals, but does not react readily with

ketones and is at best a feeble and sluggish reducing agent. Since the same effect was achieved by cyanide and by thiourea, the implication is strong that the effect was produced by the inactivation of a heavy metal catalyst. Since, in all probability, thiourea does not strongly bind iron it can be suggested that the heavy metal concerned is probably not iron but may be copper. We can conclude, therefore, that the adrenergic reaction of the smooth muscles studied requires for its operation the presence in the tissue of a heavy metal component. These experiments, of course, do not imply that the reaction catalyzed by the heavy metal must be an oxidative one nor that epinephrine itself is the substrate oxidized, though neither of these suggestions is incompatible with the results of these experiments.

TABLE 1

Substances which enhance and prolong the epinephrine response of the rabbit's intestine

CRYSTALLOIDS	CONCENTRATION	COLLOIDS	CONCENTRATION
Sodium diethyldithiocarbamate	1.10^{-6}	Dialysed lens proteins	?
Potassium ethylxanthate	1.10^{-5}	Egg albumin	1.10^{-3}
8-Hydroxy-quinoline	5.10^{-7}	Dialysed gelatine	1.10^{-4}
Sodium citrate	1.10^{-5}	Dialysed gum arabic	1.10^{-2}
Ascorbic acid	1.10^{-7}		
Glutathione	1.10^{-7}		
Tryptophane	3.10^{-4}		
Tyrosin	$\frac{1}{10}$ sat.		
Adrenalone (keto-compound to epinephrine*)	5.10^{-7}		
1(3-4-dihydroxyphenyl-) 2 aminopropanone (keto-compound to corbasil)*	5.10^{-7}		

* These two substances were kindly furnished us by the Winthrop Chemical Company.

SUMMARY

Under appropriate conditions cyanide, thiourea, and allyl thiourea can be shown to inhibit or interrupt the adrenergic response of certain smooth muscle preparations. The anti-adrenergic effect occurs with concentrations of these substances which leave unchanged the capacity of the muscle to respond to non-adrenergic stimuli. These phenomena have been demonstrated both on the rabbit's non-pregnant uterus in which the epinephrine normally excites contractions, and on the rabbit's small intestine in which epinephrine normally inhibits contractions.

It is concluded that the adrenergic response in these organs requires for its operation the presence in the tissue of a heavy metal component, possibly copper.

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SOME EFFECTS OF LOW BAROMETRIC PRESSURES ON KIDNEY FUNCTION IN THE WHITE RAT[1]

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In a recent monograph on the effect of anoxia on the body, Van Liere (1942) wrote of oxygen lack and the secretion of urine: "Since, however, anoxic anoxia at times may produce an oliguria, and at other times a polyuria, it is quite clear that more work is needed to establish clearly the mode of action of anoxia on the kidney."

Experiments reported by Van Liere and co-workers (1935) on barbitalized dogs breathing oxygen tensions from 5 to 12 per cent oxygen (equivalent to altitudes from 14,000 to 30,000 ft.) and by Toth (1937) on dogs anesthetized with dial and breathing oxygen tensions of approximately 8 per cent (equivalent to 24,000–25,000 ft.) agreed in that the majority of such animals responded to low oxygen tensions by oliguria; but occasional animals, in contrast, developed marked polyuria. Van Liere suggested that the oliguric threshold was approximately 12 per cent oxygen (about 15,000 ft.), below which the urine output was decreased.

The use of anesthetics undoubtedly influenced the above results, for in later experiments Toth (1940) reported that in unanesthetized dogs with bladder fistulae polyuria was the common response to low oxygen tension. Langley and Clarke (1942) maintained unanesthetized dogs in a low-pressure chamber at 20,000 feet altitude equivalent, and observed a polyuria on the first day which tapered off on the following days of the experiment.

Species difference appears to be as important as anesthesics in producing discordant results, for Armstrong (1939) has shown that humans subjected for 4 or 7 hours daily to a simulated altitude of 12,000 feet (483 mm. Hg) in a low-pressure chamber developed a polyuria in which the urine output was increased from 100 to 300 per cent over normal. McFarland and Edwards (1937) had previously reported that men making transatlantic flights at altitudes of 8,000 to 12,000 feet showed no urinary volume changes in the case of two observers, but a tendency toward polyuria in the airmen responsible for the handling of the ship. These conditions could not be considered controlled, however, and emotional and other influences undoubtedly were operative.

Experiments performed in this laboratory over the past 3 years on unanesthetized white rats in a low-pressure chamber have indicated that these animals respond to high altitudes in a manner corresponding to human beings rather than to dogs. Thus, while 3-hour exposure to 10,000 feet altitude equivalent pro-

[1] This investigation has been made with the assistance of grants from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association, and from the Ella Sachs Plotz Foundation.

duced no change in their urine output, exposure to 15,000 feet invariably resulted in polyuria, which, moreover, did not decrease or disappear on repeated exposure (Silvette, 1942).

Other experiments on renal function in unanesthetized white rats in low-pressure chambers will be reported below. The conditions of these experiments were chosen to simulate actual flight conditions as closely as possible. The metabolic period was 3 hours in every case, and experiments were designed to illustrate the effects of discontinuous (repeated daily) exposure rather than continuous exposure. Long or continued exposure to high altitudes without additional oxygen supply represents an entirely different problem from that investigated here.

Our experiments may conveniently be divided into the following groups:

1. Effect of various high altitudes on urinary output.
2. Influence of temperature on urine and phenolsulphonphthalein excretion at high altitudes.
3. Effect of anemic anoxia on urine output.
4. Influence of preliminary water-load on urinary excretion at high altitudes.
5. Effect of repeated daily exposure to high altitudes on urinary excretion and kidney weight.
6. Effect of post-pituitary extract on the polyuria of high altitudes.

Apparatus. Two types of low-pressure chambers were constructed, both of which were fitted with automatic devices for maintaining both constant temperature and a constant low pressure. The first was designed to hold 12 individual urine metabolism cages, but because of the reinforced wooden structure of this chamber only experiments up to 15,000 feet altitude equivalent (428 mm. Hg) were performed therein. For experiments from 15,000 feet to 30,000 feet altitude equivalent (up to 226 mm. Hg), four glass desiccators, each of 9 liters capacity, were enclosed in a miniature constant temperature room, and 3 animals placed in a divided cage in each low-pressure (desiccator) chamber. Urine was collected under paraffin oil, individually in the case of experiments up to 15,000 feet, and pooled in four groups of 3 rats in experiments at higher altitude equivalents. A continuous measured supply of fresh air was permitted to pass through the low-pressure chambers, the rate of flow being at least ten times the calculated requirements of the 12 animals comprising each single experimental group.

Results. 1. *Effect of various high altitudes on urinary output.* The average urinary output of 84 control white rats while in the low-pressure chamber but at room pressure and temperature was 0.4 cc. per 100 grams body weight per 3-hour metabolism period. The urine output at 10,000 feet altitude equivalent (523 mm. Hg) was not significantly different from the control figure, but at 15,000 feet altitude equivalent (428 mm. Hg) the average urine output rose to 1.0 cc. urine per 100 grams body weight during the metabolic period, an increase over the normal of 150 per cent. This polyuria was still more marked at an altitude equivalent of 25,000 feet (282 mm. Hg), for in 63 rats maintained at this pressure for 3 hours the average urine output was 1.6 cc. per 100 grams, or an increase of 300 per cent over the control levels.

The high-altitude polyuria could apparently be modified to a certain extent by previous exposure to lower altitudes. Figure 1-A shows the average urine output of 12 rats at room pressure (0 feet, the relatively unimportant height of the laboratory above actual sea-level being disregarded), followed by their average urinary output at 15,000 feet for the next 3 days. Figure 1-B illustrates the effect of exposing a fresh group of 12 animals to 3 daily periods at 10,000 feet *before* their first exposure to 15,000 feet. The results indicate that preliminary exposure to the lower altitude so acclimated the animals that 15,000 feet exposure no longer induced a polyuria; it was now necessary to reduce the pressure to 20,000 feet altitude equivalent before the first evidence of diuresis was observed. Raising the altitude to 25,000 feet and then to 30,000 feet in this series increased still further the observed polyuria (fig. 1-B).

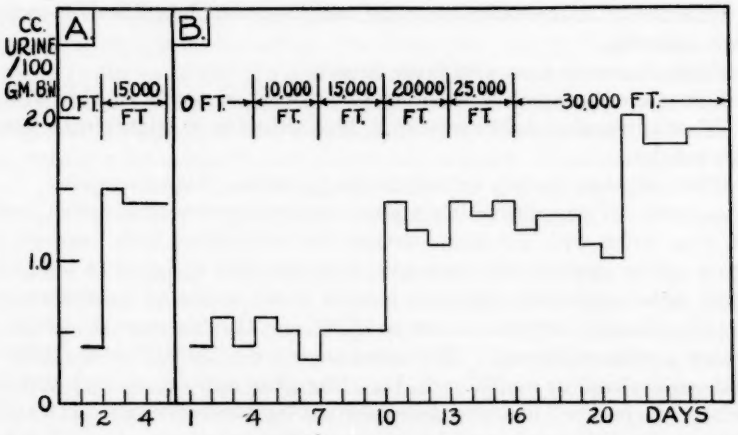

Fig. 1. Effect of 3-hour exposure to various high altitudes on urine excretion of white rats.

2. *Influence of temperature on urine and phenolsulphonphthalein excretion at high altitudes.*[2] Groups of 12 rats were injected intraperitoneally with 5 cc. per 100 grams of a solution containing 0.2 per cent sodium chloride and 2 mgm. per cent phenolsulphonphthalein and then placed in individual metabolism cages in the low-pressure chamber at an altitude equivalent of 15,000 feet. The chamber was maintained at a constant temperature of 10°, 20°, 30° or 40°C., and at the end of a 3-hour metabolic period the urine was measured and the dye determined colorimetrically. Results are given in table 1 as averages ± probable error of the average. It will be seen that, regardless of the temperature from 10° to 30°, the animals reacted to 15,000 feet equivalent altitude by polyuria, which was further increased by cold but decreased by warmth. The dye excretion, however, was insignificantly affected, though it is questionable whether a phenolsulphonphthalein excretion time as long as 3 hours has more than comparative value. When the temperature was raised to 40°, practically all of the animals died within the experimental period, and those still surviving at

[2] These results were abstracted in part in Silvette (1943a).

the end were anuric. This was probably due to the high temperature and not to the low pressure involved.

To compare the effects of 15,000 and 25,000 feet altitudes, a series of 44 animals was injected as above and maintained for 3 hours at 25,000 feet. The average urine output at 25,000 feet was 3.7 ± 0.17 cc. per 100 grams as compared with 4.0 ± 0.12 cc. at 15,000 feet (both at 20°C.). Whereas in uninjected animals the 25,000 feet polyuria was much greater than the 15,000 feet diuresis (see section 1 above), in animals carrying a water load of 5 cc. per 100 grams body weight the 25,000 feet polyuria was less than that observed at 15,000 feet.

3. *Effect of anemic anoxia on urine output.* Before investigating the further effects of anoxic anoxia (due to decreased barometric pressure) on urinary output, the comparable effects of anemic anoxia (due to the inhalation of carbon

TABLE 1

Effect of various temperatures on urine and phenolsulphonphthalein output of white rats at high altitudes

NUMBER OF RATS	TEMPERATURE OF CHAMBER	0 FEET		15,000 FEET	
		Urine output	P.S.P. excretion	Urine output	P.S.P. excretion
	°C.	*cc./100 gm./3hrs.*	*per cent*	*cc./100 gm./3 hrs.*	*per cent*
36	10	3.7 ± 0.13	66 ± 1.1	4.7 ± 0.12	65 ± 0.9
36	20	2.7 ± 0.09	64 ± 1.4	4.0 ± 0.12	70 ± 1.1
36	30	2.1 ± 0.08	55 ± 1.0	3.7 ± 0.14	61 ± 1.0
12	40	0.5		(dead)	

TABLE 2

Effect of carbon monoxide on urine and phenolsulphonphthalein excretion of rats

NUMBER OF RATS	EXPERIMENTAL CONDITIONS	URINE OUTPUT (AVERAGE ± P.E.$_{av.}$)	P.S.P. EXCRETION (AVERAGE ± P.E.$_{av.}$)
		cc./100 gm. B.W./3 hrs.	*per cent*
36	Normal control*	2.7 ± 0.09	64 ± 1.4
46	Exposed to carbon monoxide (0.4%) for 60 minutes	2.2 ± 0.08	56 ± 0.07

* See text.

monoxide) were determined. The 500-liter chamber containing 12 rats in individual metabolism cages (previously injected with fluid and phenolsulphonphthalein as in section 2 above) was sealed and partially evacuated, and the calculated amount of pure carbon monoxide gas was allowed to flow in to bring the CO-concentration of the enclosed air (now at room pressure) to 0.4 per cent. This concentration of gas was allowed to remain for 60 minutes before being replaced by fresh air. Four-tenths per cent carbon monoxide acting over one hour was sufficient to color the animals' skin (ears) the typical cherry-red in most instances, but was not lethal in any case or even seriously toxic, as judged by their general condition.

After a 3-hour metabolic period the urine was measured and the dye concentration determined colorimetrically. The results are given in table 2, compared

with control animals under similar conditions save for the absence of carbon monoxide. It will be observed that anemic anoxia brought about by carbon monoxide resulted in decreased urine and phenolsulphonphthalein output in contrast to the polyuria with normal or even increased dye excretion induced by anoxic anoxia due to exposure to low barometric pressure.

When the carbon monoxide concentration was further increased to 0.8 per cent there were many deaths, but in the still-living animals the oliguria was even more pronounced than in the case of rats exposed to 0.4 per cent concentration of the gas.

4. *Influence of preliminary water-load on urinary excretion at high altitudes.* Further experiments were designed to test the effect of various water-loads on urine output. As shown in table 3, the greater the water-load between 0 and 10 cc. per 100 grams body weight, the greater the *absolute* excretion of urine but the less the relative increase in urinary output of the animals maintained at 15,000 feet over their control (0 ft.) levels.

5. *Effect of repeated daily exposure to high altitudes on urinary excretion and kidney weight.*[3] Fresh male rats maintained on a stock diet of Purina dog chow

TABLE 3

Effect of varying water-load on excretion of urine at 15,000 feet altitude equivalent

NUMBER OF RATS	WATER LOAD	AVERAGE URINE OUTPUT AT 0 FEET	AVERAGE URINE OUTPUT AT 15,000 FEET	INCREASE IN URINE OUTPUT AT 15,000 FEET OVER 0 FEET LEVEL
	cc. 0.2 % NaCl/100 gm. B.W. i.p.	*cc./100 gm. B.W./3 hrs.*	*cc./100 gm. B.W./3 hrs.*	*per cent*
36	0	0.4	1.0	150
36	5	2.7	4.0	48
36	10	3.9	5.0	28

and liberal amounts of greens, with water allowed *ad libitum*, were used. In groups of 12 they were weighed and placed in metabolism cages in the low-pressure chamber which was then evacuated to 15,000 feet altitude equivalent for a period of 3 hours. During this time the temperature was not controlled and generally varied from 23° to 28°. At the end of the 3-hour metabolic period air was admitted, the animals removed and their urine volume read and recorded. Three groups of animals were exposed to this simulated altitude for 3 hours daily for 17, 27 and 27 days respectively, and the average urine output of the first two groups is shown graphically in figure 2. The third group was sacrificed at the end of 27 days for histological examination and for the determination of organ weights. At the same time a control group of rats, maintained for the same period of time on the same diet and under similar environmental conditions, except for non-exposure to high altitudes, was sacrificed for control observations.

Out of the total of 36 animals given 3-hour daily exposures to an altitude

[3] A preliminary report of the results at 15,000 feet equivalent altitude was given in Silvette (1942).

equivalent to 15,000 feet, only one died (on the 20th day). The remainder of the animals gained weight normally and appeared to be in perfect health throughout the course of the experiment.

The polyuria previously observed on single exposure of animals to 15,000 feet altitude was maintained throughout the long-term experiments at a generally high level. An initial marked diuresis observed in one group was not sustained (fig. 2-B), which may perhaps be taken as evidence of acclimatization; but at no time in the experiment did the urine output for the 15,000 feet metabolic period fall to that observed at room pressure. The other two groups did not show even this slight evidence of acclimatization, though in all three series the polyuric

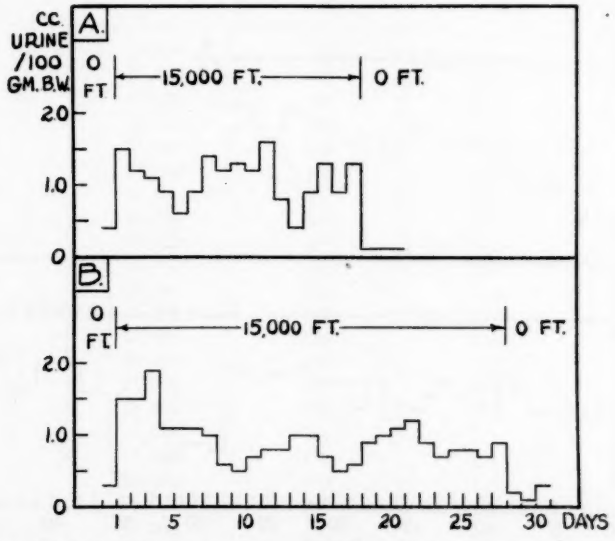

Fig. 2. Effect of daily 3-hour exposure to 15,000 feet altitude equivalent on urinary output of white rats.

response was cyclic, which may have been due to outside meteorological conditions prevailing throughout the course of the experiment.

When the animals were tested at room pressure following the determination of the period of 15,000 feet exposure, the urine output was observed to approximate that of normal animals never exposed to low pressures.

Two groups of 12 rats were similarly exposed for 3 hours daily to an altitude equivalent of 25,000 feet for 27 days each; the average urine output is shown graphically in figure 3. At the end of this time one of the groups was sacrificed for histological study and organ weights, together with a control group which had not been exposed to high altitudes. The remaining group was then followed for 8 days at room pressure and then re-exposed to 25,000 feet altitude for 3 hours daily for an additional 9 days (fig. 3-B).

There were no deaths in the first group of 12 animals exposed intermittently

for 27 days to 25,000 feet, and in the other group one animal died on the 8th day and one was sacrificed on the 27th day of the experiment. In contrast to animals exposed to 15,000 feet, which gained weight normally throughout the experiment, the 25,000 feet rats actually lost weight during the first few days of the experiment and only then began a slow moderate increase in body weight. This is demonstrated in table 4, which gives the average daily weight and average respiratory rate of the series described graphically in figure 3-A. The respiratory rate showed some evidence of adaptation and then acclimatization to the daily exposure to high altitude.

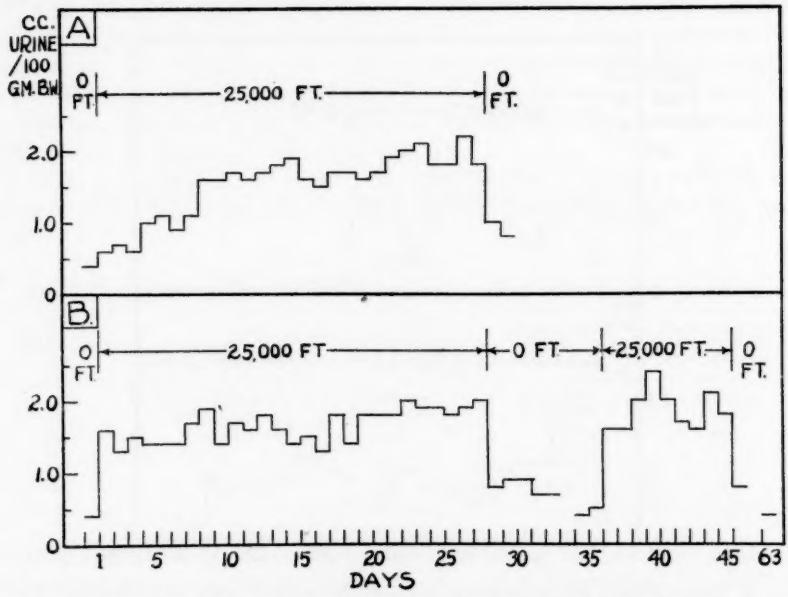

Fig. 3. Effect of daily 3-hour exposure to 25,000 feet altitude equivalent on urine output of white rats.

The polyuria observed in rats exposed daily to 25,000 feet altitude was even more pronounced than that recorded at 15,000 feet (fig. 3 and table 4). There was no evidence of acclimatization insofar as urinary output was concerned. In one group, indeed, the polyuria seemed to increase with time (fig. 3-A). Another significant difference between the two altitude effects was observable when the 25,000 feet group was tested at room pressure following the intermittent exposure to this altitude equivalent for 27 days. Immediately after long discontinuous exposure to 25,000 feet the average urinary output at room pressure was more than twice the urine excretion before the start of the experiment. Only after several days of recovery did the urine output return once more to the control level (fig. 3-B). This indicated that dysfunction in the case of the 25,000 feet group was more severe than that noted at 15,000 feet, a conclusion to which the two levels of polyuria lend contributory evidence.

Following the termination of the experiment as graphically outlined in figure 3-B, the animals were allowed to recover at room pressure under standard dietary conditions, and one month later were sacrificed for histological examination.

TABLE 4

Respiratory rate and urine output of 12 white rats exposed to 25,000 feet equivalent altitude for 3 hours daily

RUN	ALTITUDE EQUIVALENT	BODY WEIGHT (AVERAGE)	URINE OUTPUT (AVERAGE)	RESPIRATORY RATE* (AVERAGE)
	feet	*grams*	*cc./100 gm. B.W./3 hrs.*	*resp./min.*
1	0	192	0.4	78
2	25,000	190	0.6	
3	25,000	187	0.7	
4	25,000	186	0.6	
5	25,000	185	1.0	79
6	25,000	186	1.1	89
7	25,000	185	0.9	90
8	25,000	187	1.1	109
9	25,000	184	1.6	102
10	25,000	188	1.6	104
11	25,000	189	1.7	103
12	25,000	187	1.6	105
13	25,000	192	1.7	106
14	25,000	192	1.8	101
15	25,000	192	1.9	100
16	25,000	193	1.6	98
17	25,000	193	1.5	96
18	25,000	196	1.7	96
19	25,000	199	1.7	102
20	25,000	198	1.6	98
21	25,000	200	1.7	94
22	25,000	202	1.9	93
23	25,000	203	2.0	97
24	25,000	204	2.1	98
25	25,000	201	1.8	101
26	25,000	203	1.8	98
27	25,000	203	2.2	97
28	25,000	201	1.8	98
29	0	201	1.0	83
30	0	211	0.8	82

* Respiratory rates taken ½ hr. after start of each experiment, at midpoint of exp., and ½ hr. before end, and the 3 readings averaged to obtain resp. rate of each rat; thus each figure above represents average of 36 readings.

The relative weights of the kidneys in (A) normal control animals, (B) in animals exposed for 3 hours daily for 27 days to 15,000 feet altitude, (C) those of animals similarly exposed to 25,000 feet, and (D) finally those of animals exposed to 25,000 feet and allowed to recover at room pressure for one month, are given in table 3. It will be noted that the 15,000 feet exposure led to a slight degree

of relative hypertrophy and the 25,000 feet exposure to a still more marked degree, but that recovery was followed by a fall of relative kidney weight below that of the initial normal controls.

Histologically, the kidneys of rats exposed for 3 hours daily to 15,000 feet altitude for 27 days appeared quite normal except for generalized hyperemia, but signs of beginning renal damage were observed after similar exposure to 25,000 feet. In the latter animals there was also renal hyperemia, and in addition, in several instances, necrosis of some peripheral tubules in the cortex. Pathological changes in the tubules, but accompanied by ischemia in place of hyperemia, were even more evident in the kidneys of animals allowed to recover at room pressure for one month following a similar period of 25,000 feet exposure (Kindred, 1943). The red cell count of the 15,000 feet exposure animals was essentially normal, but in those exposed to 25,000 feet for 27 days the erythrocyte count rose from a normal control level of 8,700,000 per cu. mm. to 11,700,000 per cu. mm.

TABLE 5

Effect of exposure to high altitudes on kidney weight

	NORMAL CONTROLS (0 FT.)		EXPOSURE TO 15,000 FEET FOR 3 HRS. DAILY, 27 DAYS		EXPOSURE TO 25,000 FEET FOR 3 HRS. DAILY, 27 DAYS		RECOVERY PERIOD, 1 MONTH AFTER EXPOSURE TO 25,000 FEET	
	Body weight	Weight of kidneys/ 100 gm. B.W.	Body weight	Weight of kidneys/ 100 gm. B.W.	Body weight	Weight of kidneys/ 100 gm. B.W.	Body weight	Weight of kidneys/ 100 gm. B.W.
	gm.	*mgm.*	*gm.*	*mgm.*	*gm.*	*mgm.*	*gm.*	*mgm.*
Maximum	300	965	275	1050	250	1,270	320	825
Minimum	210	770	225	850	160	890	249	680
Average	*253*	*875*	*239*	*945*	*205*	*1,070*	*294*	*770*
No. rats	15	15	11	11	12	12	8	8

6. *Effect of post-pituitary extract on the polyuria of high altitudes.*[4] Experiments were performed to determine whether the polyuria observed at 15,000 feet could be reversed by the administration of post-pituitary extract. In one series of fully-fed animals injected with 5 cc. 0.2 per cent sodium chloride solution per 100 grams body weight, the 3-hour urine excretion at room pressure averaged 2.1 cc. per 100 grams. Animals similarly injected but exposed for 3 hours to 15,000 feet excreted on the average 5.0 cc. urine per 100 grams. Other animals, similarly treated but with the addition of post-pituitary extract (1.5 U.S.P. units per 100 grams B.W.) before exposure to 15,000 feet, averaged only 1.9 cc. urine in the 3-hour period, thus indicating that the polyuria of high altitudes was inhibited by the antidiuretic hormone.

Further animals were treated as follows with weekly rest periods between experiments: After a preliminary fasting period of 12 hours during which the animals were allowed water *ad libitum*, they were injected with 10 cc. per 100 grams 0.2 per cent sodium chloride solution with or without pituitary extract, and 3

[4] These results have been given in abstract by Silvette (1943b).

hours later exposed in metabolism cages either to room pressure or to 15,000 feet altitude equivalent for a period of 3 hours. Results are given in table 6. In these experiments too the injection of post-pituitary extract reversed the high-altitude polyuria. Furthermore, the water retention brought about by equal doses of post-pituitary extract either at 0 or 15,000 feet was almost the same, i.e., 3.2 cc. at room pressure and 3.3 cc. at 15,000 feet. This would seem to indicate that the renal tubules were as sensitive to the antidiuretic hormone at low barometric as at room pressure.

DISCUSSION. Exposure to sufficiently high altitudes for 3-hour periods invariably led to polyuria in the white rat. At 10,000 feet altitude urinary excretion remained unaffected, as it did at 15,000 feet if the animals were first acclimated by preliminary exposure to 10,000 feet. But fresh animals exposed to 15,000, 20,000, 25,000, or 30,000 feet showed a marked diuresis which increased as the barometric pressure was lowered. In view of the reported results of other workers (Van Liere *et al.*, 1935; Toth, 1937) that dogs subjected to low oxygen tensions developed oliguria, it was at first thought that if the barometric pressure were sufficiently lowered, rats also would respond by reduced urinary excretion.

TABLE 6

*Effect of post-pituitary extract on high-altitude polyuria**

NUMBER OF RATS	POST-PITUITARY EXTRACT INJECTED	AVERAGE 3-HOUR URINE OUTPUT AT 0 FEET	AVERAGE 3-HOUR URINE OUTPUT AT 15,000 FEET
	units/100 gm. B.W.	*cc./100 gm. B.W.*	*cc./100 gm. B.W.*
36	0	3.9	5.0
36	1	0.7	1.7

* For details, see text.

However, rats exposed even to 30,000 feet altitude equivalent for 3 hours and dying of the exposure continued to exhibit the characteristic polyuria almost to the point of death. In other words, only moribund animals showed oliguria at high altitudes.

The polyuric effect of anoxic anoxia is entirely different from the effect on urine output observed in other types of anoxia. In anemic anoxia, due to carbon monoxide poisoning, the urinary output was reduced 18.5 per cent and the phenolsulphonphthalein excretion was reduced 12.5 per cent below the control levels, whereas anoxia due to low barometric pressure caused respective increases over the normal of 48 and 9.4 per cent. The stagnant type of anoxia, resulting from hemorrhage or circulatory failure, also leads to oliguria, as clinical experience demonstrates; and so does histotoxic anoxia brought about by cyanide poisoning (Beck, Kempton and Richards, 1938). Thus, of the four types of anoxia in Barcroft's classification, only anoxic anoxia leads to polyuria.

The water loss by way of the kidneys was so severe (1.0 to 1.6 cc. per 100 grams body weight in 3 hrs., exposure to 15,000–25,000 ft.) that it could not be expected that prolonged exposure to high altitudes would bring about an even greater diuresis. Indeed, the contrary is paradoxically true, for Collings *et al.*

(1943) report that young rats exposed for 12 to 24 hours to 380 mm. Hg (18,000 ft.) had urine volumes which were essentially identical with the controls. It is likely that the initial polyuria of the first few hours, which has been the invariable reaction shown by our animals, was compensated for by a subsequent oliguria, so that the urine volume over the 12–24 hour period was apparently normal. The results of certain of our own experiments lend credence to this belief: 36 rats were primed with 10 per cent of their body weight of fluid and 2.5 hours later were placed in metabolism cages for 9 hours. Urine output for the first 3 hours (experimental period) averaged 3.9 cc. per 100 grams body weight; for the second 3 hours (first recovery period), 1.1 cc.; and for the third 3 hours (second recovery period), 0.9 cc. Later, the same rats were similarly treated, except that they were exposed to an equivalent altitude of 15,000 feet during the 3-hour experimental period. The average urine output per 100 grams body weight during this experimental period was 5.0 cc. while at 15,000 feet, then 0.5 cc. during the first and 0.6 cc. during the second recovery periods. Thus it will be seen that, while the animals really showed the characteristic high-altitude polyuria when the two experiments were compared in detail, on the basis of the *total* 9-hour urinary excretion the controls excreted 5.9 cc. urine and the high-altitude rats only 6.1 cc., essentially identical figures.

The significance of the experiments on repeated exposure to high altitudes lies in three facts: that the polyuria was maintained for the duration of the experiments essentially unchanged; that following the termination of the period of exposure the urinary excretion returned to normal levels immediately or at the longest after a few days; but at the same time there was histological evidence of limited renal damage.

The increase in kidney weight relative to body weight after long discontinuous exposure to high altitudes was apparently not due to hypertrophy of any one part of the kidney at the expense of the other parts. The renal cortex and medulla retained their normal proportions to one another, and the tubules and glomeruli their normal diameters (Kindred, 1943).

While these differences in kidney weight reported herein are statistically highly significant, it must be conceded that the high-altitude hypertrophy and succeeding atrophy of recovery can be mathematically explained on the basis of loss or gain in body weight. However, from other considerations (such as the relation of kidney to body weight in normally-growing animals (Donaldson, 1924) and detailed histological studies in progress by Kindred (1943)), it is believed that the kidney weight changes observed bear a truly valid relationship to the functional changes (in urinary output) reported, though this relationship cannot be elucidated at the present time.

Other investigators have reported that long exposure to low barometric pressures led to a decrease in relative kidney weight. Sundstroem and Michaels (1942) noted a consistent decrease in the weights of kidneys of rats exposed continuously to pressures of 460 mm. (13,000 ft.), 360 mm. (19,000 ft.), 300 mm. (23,500 ft.) and 260 mm. Hg (27,000 ft.). Tornetta *et al.* (1943) reported that male rats exposed to pressures of 250 to 280 mm. Hg (25,000–28,000 ft.) for 6

hours daily for 14 to 18 days showed significant decreases in kidney weights. Eighteen to 20 hours' exposure for 3 to 12 days led to even greater kidney weight loss.

These experiments differed from those reported herein in that the daily exposure to high altitudes was much longer, or even continuous, whereas the number of days was usually less. Since the technique of measuring relative weights of such organs as the kidneys cannot be questioned, the discordant results obtained by the various workers must be a function of the different experimental conditions set up.

The reason for the high-altitude polyuria, like the explanation of the gain or loss in relative kidney weight, is still not clear. The relative glomerular hyperemia may be active, i.e., due to afferent arteriolar dilatation; passive, i.e., due to efferent arteriolar constriction; or secondary to polycythemia, though the latter was found only at 25,000 and not at 15,000 feet. This hyperemia may well have increased glomerular filtration somewhat, but the increase in urine excretion of 300 per cent over the normal at 25,000 feet was too great to be explained except by decreased tubular reabsorption as well. That there actually was tubular damage sufficient to bring about such decreased tubular reabsorption of fluid may be inferred from the histological evidence presented by Kindred (1943) and summarized in section 5 above.

SUMMARY

Exposure to sufficiently low barometric pressures for 3-hour periods invariably led to polyuria in the white rat. After exposure to 15,000 feet altitude equivalent the urine output was increased 150 per cent and after 25,000 feet exposure 300 per cent over the control (0 ft.) levels.

The high altitude polyuria was further increased by cold (10°C.), but decreased by heat (30°C.).

Phenolsulphonphthalein excretion was not significantly affected during exposure to 15,000 feet altitude equivalent.

In contrast to the polyuria with normal phenolsulphonphthalein excretion observed at high altitudes, anemic anoxia due to carbon monoxide poisoning resulted in decreased urine and dye excretion.

The greater the water-load, the greater was the absolute excretion of urine at 15,000 feet, but the less the relative increase in urinary output over the control (0 ft.) levels.

Repeated 3-hour daily exposures of white rats to simulated altitudes of 15,000 or 25,000 feet had no significant effect on their polyuric response, which was well sustained throughout the 27-day experiments. At the end of this time, when tested at 0 feet once more, the urinary excretion was normal or returned to normal within a few days. Relative kidney weights of these animals indicated a slight degree of renal hypertrophy after the period of 15,000 feet exposure, a more marked degree of hypertrophy after 25,000 feet exposure, but an apparent atrophy after a one-month recovery period at room pressure. The histological

picture of the kidneys of animals after long intermittent exposures to 15,000 feet was normal, except for marked hyperemia, but at 25,000 feet beginning slight pathological changes occurred.

The polyuria of high altitudes may be inhibited or reversed by the injection of post-pituitary extract.

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THE EFFECTS OF LOW BAROMETRIC PRESSURES ON THE STRUCTURE OF THE KIDNEYS OF THE WHITE RAT

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Silvette (1) in a recent paper reported a greater output of urine in rats subjected for three hours daily for 27 days to low barometric pressure (428.8 mm. Hg, equivalent to altitude of 15,000 ft.) than in the same rats and in control groups maintained at room pressure. Since this publication, Silvette has carried his experiments further and has subjected rats to lower barometric pressure (equivalent to 25,000 ft. altitude) for the same periods of time. He reports a further increase in urine output as compared with the rats at room pressure (fig. 1) and at pressure equivalent to 15,000 ft. altitude (2). Silvette observed that accompanying the increased urine output there was a relative increase in the weight of the kidneys per 100 grams of body weight. Since no histologic studies have appeared concerning the kidneys of animals so treated, the animals were turned over to me following these experiments with the request that the kidneys be examined for morphologic changes which could be related to the functional changes and the changes in weight of the kidneys which were reported. The important physiologic change to which the experimental rats have been subjected is anoxia.

MATERIALS. Three groups of living rats, all males, of Wistar strain, but not litter mates, were turned over to me for study immediately after the experiments. Group I, controls, 15 rats, average weight 250 grams (range, 210–300 grams); group II, 11 rats which had been exposed to barometric pressure equivalent to 15,000 ft. altitude for 3 hours daily for 27 days, average weight 236 grams (range, 230–275 grams); group III, 12 rats which had been exposed to barometric pressure equivalent to 25,000 ft. for 3 hours daily for 27 days, average weight 205 grams (range, 160–250 grams). These rats were killed with ether. Blood counts were made from the femoral artery just before death. Following death the kidneys and certain other organs were removed and weighed to the nearest centigram.

The kidneys were either split in the sagittal axis or transverse to the long axis and fixed in either Helly's fluid or in 10 per cent formalin. The usual paraffin technic of embedding and sectioning was followed. Sections were cut at 7 microns and stained either with hematoxylin and eosin or with Kornhauser's quadruple stain (3). The latter is an excellent stain, because the red blood corpuscles, tubules and connective tissues are sharply differentiated from each other.

In addition to this material, eight male rats, average weight 294 grams (range, 249–320 grams), which had been exposed 3 hours daily for 27 days to 282 mm. Hg pressure (equivalent to 25,000 ft. altitude) following which they had been

kept at room pressure for one month, were killed by Doctor Silvette and the kidneys were removed, weighed and fixed in 10 per cent formalin and given to me. The histologic technic described above was applied to these kidneys. These will be designated as rats of group IV, and the others as groups I, II and III, respectively, in the following description.

Observations. The average weights of the kidneys per 100 grams of body weight with their standard errors were as follows: Goup I (controls); 875 ± 15 mgm. Group II (15,000 ft.); 945 ± 19 mgm. Group III (25,000 ft.); 1070 ± 36 mgm. Group IV (one month after 25,000 ft.); 770 ± 17 mgm.

The data on the weights of the kidneys of the rats of groups I and II have been used by Silvette (1) in support of his view that increased urine output is accompanied by relative increase in weight of the kidney; and those of groups III and IV in a paper in press (2). Statistical contrasts showed that all weights

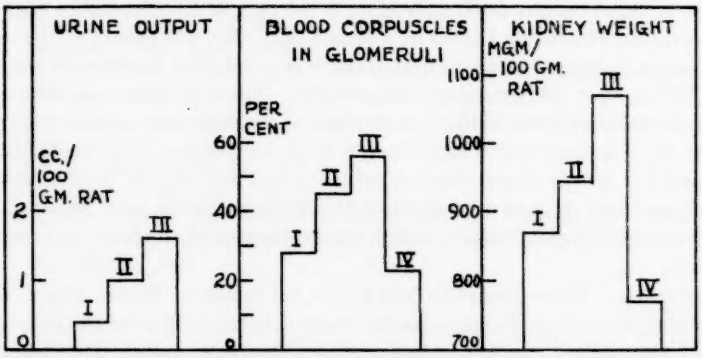

Fig. 1. Graph showing correlation between average urine output per 100 grams of body weight; average kidney weight per 100 grams body weight; and average per cent of red blood corpuscles per glomerulus of kidneys of rats maintained at barometric pressures corresponding to different altitudes. I, 0 ft., (controls); II, 15,000 ft.; III, 25,000 ft.; IV, 0 ft., one month after exposure to 25,000 ft. Data on urine output from Silvette (2).

are significantly different from each other and that weight increases as the barometric pressure decreases. The kidneys of the rats of group IV showed a very great decrease in relative weight when contrasted with the kidneys of groups II and III, and a significant but relatively lower weight than the kidneys of the rats of group I.

Examination of the sections of the kidneys revealed certain qualitative and quantitative morphologic changes which are thought to be relevant to the changes in the weights of the kidneys and to the functional polyuria observed in the experimental groups. Low magnification studies were made of sections of the kidneys of groups I and II in which the proportions of areas of cortex and medulla in a segment from approximately the same region of the kidneys were compared. No differences were observed in the relative amounts of cortex and medulla in the two groups. Counts of the glomeruli per unit volume and measurements of the sizes of the glomeruli were also made on the kidneys of these

two groups of rats using higher magnification and no differences were found. The average diameters of the tubules were found to be the same in both groups. Since the method of making these measurements is rather time consuming and since the results were negative, the measurements were not done on the rats of groups III and IV.

During these examinations of the kidney with the higher magnification (1200 diameters) it was observed that while it was quite difficult to see the intertubular capillaries in the cortex of the control kidneys (Bensley, 4, has recorded the same conclusion from study of rabbit kidneys), these capillaries were quite easily visible by reason of their distention with red blood corpuscles in the kidneys of the rats of groups II and III. In these kidneys the "vascular rays" of Bensley (4), the terminations of the efferent arteriolae rectae of the glomeruli, stood out more sharply and seemed to contain more blood than similar channels in the kidneys of the controls. Since these arteriolae rectae are directly connected with the glomerular capillary network these regions were next examined. Casual observation suggested that there was hyperemia and distention of the capillaries in the glomeruli of the kidneys of groups II and III when these were contrasted with those of groups I and IV. Such observations led to the application of a quantitative method for measurement of these conditions. The paper weight method which Jackson (5) used in estimating the comparative proportions of the stroma and parenchyma of the adrenal glands was applied. The outline of a median vertical section of a glomerulus was drawn with camera lucida on paper. Within the glomerulus the areas occupied by red blood corpuscles within the glomerular capillaries were outlined. Then the outlined glomerulus was cut out and weighed to the nearest centigram, following which the areas outlining the blood corpuscles were cut out and weighed. The percentage of red blood corpuscle volume per glomerulus was estimated from these weights. In order to see if this were a representative sample of the volume of the red blood corpuscles in the whole glomerulus, the average percentage of red blood corpuscle weight measured in serial sections of 10 glomeruli was compared with the average value obtained in only the midvertical sections of these glomeruli. The average weight of the red blood corpuscles for the whole glomeruli was 23 $\pm$ 1.9 per cent of the glomerular weight, and for the midvertical sections, 23 $\pm$ 1.7 per cent. From this contrast it was felt that the measurements of the midvertical sections gave an accurate measurement of the total weight of the red blood corpuscles in the glomeruli.

The results of these measurements are summarized in table 1, in which the percentages of red blood corpuscle weight in the glomeruli are tabulated by percentage classes in the different groups of rats. Examination of table 1 shows that in group I (controls) the modal classes are those in which the red blood corpuscles occupy between 21 and 40 per cent of the glomerulus; in group II (15,000 ft.) the modal classes extend from 31 to 50 per cent; in group III (25,000 ft.) from 51 to 70 per cent; and in group IV (one month after 25,000 ft.) from 11 to 30 per cent. This table shows that by the method used there is evidence of an increase in the amount of red blood corpuscles in the glomeruli

with decrease in barometric pressure and that when the rats are removed from this environment and kept at room pressure for one month there is a sharp drop in the amount of blood present.

Further statistical analysis in which the means of the blood corpuscle percentages with their standard errors of the several groups were contrasted shows the following results: Group I, average blood corpuscle percentage 28 ± 0.65 per cent. Group II, average blood corpuscle percentage 45 ± 1.3 per cent. Group III, average blood corpuscle percentage 56 ± 0.81 per cent. Group IV, average blood corpuscle percentage 23 ± 0.93 per cent.

Difference between groups I and II, 17 ± 1.48 per cent; t 11.5. Difference between groups II and III, 9 ± 1.5 per cent; t 6.0. Difference between groups I and IV, 5 ± 1.12 per cent; t 4.45.

TABLE 1

Distribution by classes of percentage of red blood corpuscles in the glomeruli of kidneys of control rats and of rats exposed to low barometric pressures

I, controls; II, 15,000 ft.; III, 25,000 ft.; IV, one month after 25,000 ft. The percentages of the red blood corpuscles were measured in midvertical sections of ten glomeruli from each kidney; number of kidneys: I, 10 from 10 rats; II, 9 from 9 rats; III, 11 from 11 rats; IV, 8 from 8 rats. Total number of rats, 38; total number of kidneys, 38; total number of glomeruli, 380.

PER CENT	GROUP			
	I	II	III	IV
1–10	0	0	0	2
11–20	16	0	0	33
21–30	49	1	0	30
31–40	32	30	6	15
41–50	3	38	24	0
51–60	0	17	45	0
61–70	0	4	34	0
71–80	0	0	1	0

Since the t ratio in all these contrasts is larger than 2.5, the differences are considered to be greater than could occur by chance and hence are statistically significant (6). These data establish the fact that changes in the environmental barometric pressure produce measureable effects on the amount of red blood corpuscles in the glomerular capillaries.

Such changes in the amount of blood corpuscles could very well account for part of the increased diuresis which accompanies the exposure if the results are interpreted according to the suggestion by Drinker and Yoffey (7) which is as follows: "It is conceded that capillaries in most places in the body experience change in diameter and that these changes constitute an important regulatory feature of many physiologic reactions such for example as muscle work, formation of urine, etc." In this particular group of experiments the capillaries in the glomeruli of the kidneys of the experimental rats are distended with red blood corpuscles and such distention could accordingly lead to increased glomerular

capillary pressure and increased capillary permeability, an interpretation which is suggested by the conclusion of Landis (8) that permeability of the endothelium of the capillary is not determined by the capillary diameter, but by capillary pressure.

It has been suggested by Richards and Schmidt (9) that the blood flowing through the glomerular capillaries is controlled by an efferent constrictor mechanism and it has been shown by Bensley (4) in the rabbit and guinea-pig kidneys that there are cells (pericytes) arranged in circular fashion around the efferent arterioles which conceivably could perform this function. Hence the sections of the hyperemic kidneys were examined to see if the hyperemia of the glomeruli could have been produced by contraction of such cells. Serial sections of glomeruli were examined and the efferent arterioles were found to be widely dilated and directly continuous with the enlarged intertubular capillaries. Hence the dilatation of the capillaries in the glomeruli can only be viewed as one effect of a relaxation of the whole renal vascular system. For not only were the intertubular capillaries enlarged, but the archiform veins were also more distended than were those in the kidneys of the control rats. Hyperemia, then is characteristic of the whole kidney and since it is present in rats which have been exposed to anoxic conditions it may logically be concluded that the hyperemia is the result of the anoxia, but whether it has been brought about by local tissue response or through a central mechanism cannot be decided by the evidence presented here. Furthermore, it seems logical that the polyuria characteristic of these rats is partly the result of increased glomerular pressure and greater capillary permeability, although as Silvette (2) has suggested, the observed polyuria was too great to have been the result of increased glomerular pressure alone. The correlation between the output of urine and the percentage of red blood corpuscles in the glomeruli is shown in figure 1. No statistical analysis was made of this correlation, hence it can only be suggestive.

Another view which might be taken is that the rats have been so affected by the anoxic conditions that there has been an increase in the number of red blood corpuscles per cubic millimeter and that the hyperemia observed in the glomeruli is the result of anoxic polycythemia. An analysis of this possibility showed that neither in group I (controls, ave. rbcs. per cu.mm., 8.65 millions) nor in the rats of group II (15,000 ft., ave. rbcs. per cu.mm., 7.7 millions) is there any correlation between glomerular hyperemia and the rbc. count. In the rats of group III (25,000 ft., ave. rbcs. per cu.mm., 11.7 millions) there is a suggestive positive correlation, but it is not considered statistically significant. Therefore, it is concluded that such polycythemia as may occur in the rats of group III is not sufficient to account for the renal hyperemia observed.

As has been stated in the introduction, Silvette (1) observed that increase in relative weight of the kidneys was accompanied by increased output of urine. The question might now be asked whether there is any correlation between hyperemia and the weight of the kidneys. Statistical analysis of this question, using the kidney weight per 100 grams of body weight and the percentages of red blood corpuscles in the glomeruli as the contrasting values, has shown that

for the whole population, pooling the weights of the kidneys of rats in all groups, the moment of correlation as derived using the methods of Fisher (6) is 0.655. Such a value implies that the correlation is significant of an association which is better than could occur by chance. This correlation is shown in figure 1. Since the glomerular hyperemia is one part of the total renal hyperemia which is characteristic of the kidneys of the experimental rats it is possible to conclude, using the established correlation as an indicator only, that the observed increase in kidney weight in rats exposed to low barometric pressures could be accounted for by the additional blood present.

In addition to the changes which can be called normal, it was observed that in the kidneys of the rats of group II (15,000 ft.) the histology of the kidney was unchanged except for the hyperemia. In the kidneys of the rats of group III (25,000 ft.) damage appears to begin. As in the rats of group II, the predominant picture is one of hyperemia of the glomeruli, intertubular capillaries and veins. However, in some of the kidneys there were small areas of necrosis of tubules in the cortex and one kidney showed a small area of tubule and glomerular shrinkage. In kidneys of six of these rats there were small areas of lymphocyte infiltration, but since similar areas were found in some of the kidneys of the control group, this condition is not considered to be significant. In one kidney of one rat there was an accumulation of neutrophilic granulocytes in the hilar fat subjacent to the renal sinus mucosa.

In the kidneys of the rats of group IV, the necrosis observed in some of the kidneys of rats of group III was present in kidneys of five of the eight rats studied. There was shrinkage and flattening of the glomeruli and peripheral tubules in some parts of the cortex in the kidneys of all of these rats. The kidneys were extremely ischemic and no blood was seen in the intertubular capillaries or in the vascular rays. As has been pointed out above the glomeruli were notably deficient in blood content. Calcareous deposits were present in the tubules of the cortex of one kidney. In four of the eight kidneys, neutrophilic granulocytes were massed in the hilar fat subjacent to the renal sinus mucosa. There was fibrosis in the region of the vascular rays in one kidney. On the whole, the kidneys of this group showed distinct pathologic changes when contrasted with the kidneys of the other groups.

From these observations on kidney damage it may be concluded that the kidneys of rats can stand the anoxia when subjected to it, but the degree of anoxia (concomitant with lowered barometric pressure) produces a latent condition of parenchymatous damage which appears to become extensive after the rats have been removed from the anoxic condition for some time. More study should be given to this effect as the possibility of permanent kidney damage resulting from chronic exposure to anoxic conditions whether in industry or in aviation has not been realized.

SUMMARY

The kidneys of rats which had shown polyuria while exposed to low barometric pressure daily for 3 hours for 27 days (1, 2), show relative increase in weight,

hyperemia and a slight degree of parenchymatous damage. There is a positive correlation between hyperemia and the increase in weight of the kidneys. It is suggested that diuresis is related in part to increased permeability of the glomerular capillaries because of the increased glomerular pressure assumed to accompany capillary distention. The direct effect of anoxia (which accompanies the low barometric pressures) on the kidney vasa is suggested as the cause underlying hyperemia.

The kidneys of rats which have been returned to room pressure for a month following the exposure to low barometric pressure show extensive parenchymatous damage, calcification, shrinkage of the stroma, and ischemia.

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THE VALIDITY OF "OVULATION POTENTIALS"[1]

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An increase in the difference of potential between a vaginal and a suprapubic (surface) electrode at the time of ovulation was first noted by Burr, Hill and Allen (1) in the rabbit. This finding was confirmed by Reboul, Friedgood and Davis (2, 3). In the rat similar electric changes at the time of estrus were observed by Rogers (4–6). Employing surface electrodes on abdominal and flank areas respectively, Altmann (7, 8) reported marked voltage gradients in the hen at the time of ovulation, and in the sow simultaneously with external signs of estrus.

In the human female, Burr et al. (9, 10), using an abdominovaginal circuit, described the same phenomenon during ovulation time in one case as did Rock, Reboul and Wiggers (11) also in one patient. In 1938, Rock, Reboul and Snodgrass (12) repeated these experiments on nine other women during the theoretical ovulation period (based on catamenial history). In some of these experiments, however, a pair of surface electrodes, applied at various levels between the umbilicus and the symphysis pubis was used, alone, or in conjunction with a second surface pair, or with an abdominovaginal circuit.

When the pertinent data of all ten cases of Rock and his associates (11, 12) were analyzed, a number of inconsistencies were noted in the results obtained. In the first place, regardless of the type of set-up: whether abdominovaginal electrodes or surface electrodes were used, or whether both types of circuit were employed simultaneously, the potential change was noted in some cases even before ovulation had occurred. Therefore a statement was made in the original report (12) that the change was only *associated* in some way with ovulation.

In the second place, there were wide and inexplicable variations in the magnitude, duration and polarity of the electric changes.

Furthermore, unaccountably erratic results were obtained when surface electrodes were employed simultaneously with an abdominovaginal circuit. In one case, definite changes were recorded in the surface circuit in the absence of any change in the abdominovaginal system.

If these "ovulation potentials" are all due to electric activity in the ovary associated with the process of ovulation (3), it is difficult to see how the potential changes can increase in magnitude as the electrodes are moved further away from the ovary. In one patient tested, the change in potential between a pair

[1] Aided by grants from the American Academy of Arts and Sciences, the William F. Milton Fund of Harvard University, the Committee for Research in Problems of Sex, National Research Council, and Mr. Richard C. Paine.

of surface electrodes placed low in the pelvis was approximately three times as great in magnitude as that between the abdominovaginal pair. Moreover, in all but one case, the highest values obtained were those recorded from surface electrodes.

An increase in magnitude of the difference in potential as the electrodes were moved away from the ovary was also noted by Reboul, Davis and Friedgood (3). In addition, Rogers' (6) finding of a similar change in difference of potential on the last day of estrogen-induced estrus in the spayed rat further increased our skepticism as to the relation between the so-called "abdominovaginal sign" and ovulation. It seemed evident that some hitherto unknown factor must be concerned in the production of these widely variable results.

In order to ascertain the nature of this factor, it was deemed essential, first of all, to determine whether one could locate a point on the surface which would be stable in potential and could thus serve as the site for a reference electrode. With this purpose in mind, the abdominal region and thighs of six human subjects were mapped in terms of electric potential.

I. Skin potentials in relation to the "abdominovaginal sign" of ovulation. *Surface Potentials: Isopotential Contour Patterns. Method.* The reference electrode consisted of a large Ag-AgCl plate about 45 sq. cm. in area. This was firmly fastened just above the left knee by means of a loose elastic binder. A layer of cotton soaked in physiologic saline solution was placed between the chlorided silver sheet and the skin. The exploring electrode, also of Ag-AgCl, had an effective contact area of about 16 sq. mm.; here, too, a small piece of cotton moistened with physiologic salt solution made the actual contact with the skin.

The difference in potential between the electrodes was measured by means of a null method. The effective input resistance to the measuring circuit was 2 meg. ohms. The null point was usually quite sharp, and currents less than 5×10^{-10} amp. flowed in the electric circuit. The unbalanced potential was shunted by a vibrating contact at the grid of the first tube of a high-gain audio frequency amplifier. The output of the amplifier operated a pair of sensitive head-phones. Fairly accurate potential determinations could be made in 3 to 5 seconds.

Results. On each individual studied, 75 to 100 determinations of potential were made. When the potentials were sufficiently stable, it was possible to construct a clearly defined series of isopotential contours, as shown in figure 1. No demonstrable effect of sex on isopotential contours was noted.

In general, a focus of potential may be found on the thighs in the region where the femoral artery and vein are most superficial. Potential foci may be observed also at almost any point on the abdomen and do not seem to have any demonstrable connection with underlying organs.[2] As a rule, there are relatively few or no potential foci in the suprapubic area.

[2] On one individual a very interesting observation was made which tends to indicate further the cutaneous nature of these potentials. In plotting the isopotential contours of any subject, the potential value of a particular area was written on the skin by means of a stub-pointed fountain pen, since the latter required less pressure than the usual skin pencil and caused little or no disturbance in the potentials. One subject happened to exhibit marked dermographia, and it was strikingly clear that the more positive the potential of the area, the higher were the wheals produced by the pen-point.

On certain individuals, potential foci and isopotential contours were very stable and the same pattern was obtained at intervals as great as two weeks. Other individuals had such unstable potentials that even a small abdominal area could not be plotted. In general, persons tending toward the "sthenic" type seemed to be unsuitable as subjects for isopotential contour plotting.

In three subjects, measurements were made during the estimated ovulation time while they were awaiting operation. If ovulation is accompanied by a fairly large electric potential change in the ovary, such a shift in voltage might reasonably be expected to appear in the isopotential contour pattern. Yet even

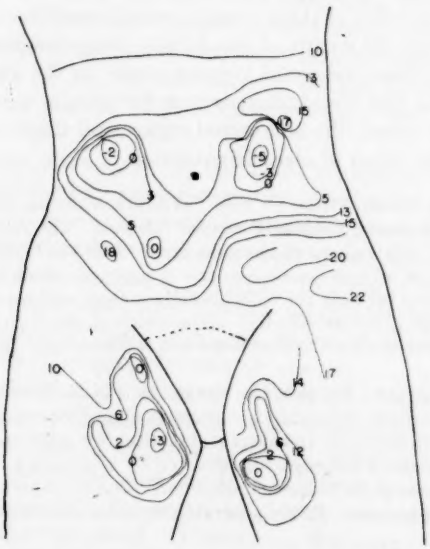

Fig. 1. Isopotential contours measured on a male subject. Potentials plotted in millivolts. For measuring purposes a large reference electrode was placed just above the knee. Potentials plotted with respect to arbitrary value of zero for potential focus in left femoral region. The voltage range shown is from +22 mv. to −5 mv. Note the steep potential gradient near the lower left boundary of the epigastric region. The potential ranges from −5 to +17 mv. within an actual distance of 4 cm.

in the presence of electric instability, no clear potential foci were found which could be related to the underlying ovaries. As far as could be determined, the reason for the instability of the electric potential was due entirely to the emotional uneasiness of the subjects.

Direct Measurements of the Ovary. Still further to check the ovary as a possible source of potential, direct electric measurements were made on human ovaries *in situ* of three women during the course of routine laparotomy. In each case the operation was performed within the patient's estimated ovulation time.

Method. The same leads and measuring equipment as described above were used, except that for the present experiments we devised a special unipolar exploring electrode of Ag-AgCl agar which could be autoclaved. This was made by plugging an 8-inch length of $\frac{1}{16}$-inch glass tubing at one end with a loose roll of filter paper about $\frac{1}{2}$ inch long. The tube was filled with a hot agar-saline solution, and a 4-inch length of no. 20 B. & S. gauge chlorided silver wire was pushed down into the agar. The silver wire was soft-soldered to a fine multi-stranded copper wire which, in turn, was pulled through an 8-foot length of $\frac{1}{4}$-inch pure gum tubing ($\frac{1}{16}$-inch bore). The lead was fastened to the top of the electrode tube by means of rubber bands. The electrode and its lead wire were inserted in a 10-inch glass cylinder. The latter was plugged with gauze in the usual manner, and the assembly placed in the autoclave in an upright position. For convenience, the potential determinations were referred to the broad ligament as zero.

Results. In general, no potentials greater than ± 1 mv. were found between any two organs in the abdominal cavity. Several follicular surfaces (including that of one fairly large, presumably mature follicle[3]), as well as the intervening ovarian cortex, were tested, but no significant electric potential was detected. The highest potential recorded, slightly less than $+2$ mv., was obtained from a locus on the *Rectus abdominis* muscle.

II. Factors influencing "finger-to-finger potentials."[4] Several investigators have recorded an increase in the difference of potential between the index fingers of women at various times during the menstrual cycle, and have concluded that such a change indicated ovulation (14–19). Barton (17) deduced from her data that follicular rupture might take place at any time, even during the menses, and sometimes twice in the same cycle, while Altmann (19) reported that ovulation might occur between the 5th and 23rd days.

Since the establishment of a clearly defined criterion of ovulation time would be most important, both from a theoretical and from a clinical viewpoint, we undertook in 1939 similar experiments on 30 nurses (group 1) and 23 female employees (group 2) of the Free Hospital for Women. In addition to women whose cycle lengths were within normal limits, the subjects included also one surgical castrate, one woman in natural menopause, one pregnant individual, and two women who, on the basis of their clinical history, doubtless had anovulatory cycles (20).

With the exception of Sundays, measurements were made daily before lunch on all subjects during the months of April and May. In addition, one of the two groups of subjects was also studied during the last week of March.

It should be noted that, in order to rule out any factors which might unconsciously influence the experimenter, the latter knew nothing about the menstrual dates or clinical history of the subjects. At the beginning of the experimental period, the women were all given printed calendar cards on which to record their catamenial dates and any other pertinent information. The cards were collected by the investigator only at the conclusion of the experimental period.

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[3] At present we know of no reliable way to determine by gross examination alone whether a large follicle is normal or atretic.

[4] A preliminary report of this work was presented before the American Physiological Society, New Orleans, 1940 (13).

Method. The potential determinations were made with the null-measuring system described above.[5]

Experience with meter-indicating types of vacuum tube voltmeters shows that it is frequently almost impossible to determine whether or not a particular meter reading is a true indication of the real potential. Occasionally the readings have been found to be in error by several hundred per cent. This may be due to several causes, the most important of which is the difficulty of shielding the subject completely from stray 60-cycle electric fields. Radio frequency fields may be particularly troublesome, as some element in the external circuit, or the vacuum tube itself, may rectify the high frequency and produce deflections of the meter. This is especially true in hospitals where diathermy machines are employed. The particular type of null measuring equipment used to obtain the data presented in this paper possessed the advantage of giving an immediate acoustic clue as to the probable source of spurious potentials, and hence proper precautions could be taken in order to obtain a correct determination of voltage. The measuring equipment was also free from the erratic and disturbing influences of electrostatic charges, however generated.

The fingers were immersed in beakers of saline solution, in the manner described by Burr and Musselman (14). However, unlike investigations of other workers, where measurements were confined to the index fingers, determinations in the present experiments were made between *each* pair of fingers, excluding the thumb; that is, pairs of index fingers, middle fingers, etc., were tested separately. The potential readings from the four pairs of determinations were averaged for each subject, and this individual mean was used in calculating the average for each of the two large groups (group 1, nurses; and group 2, employees).

Finger Potentials in Relation to the Presence or Absence of Normal Menstrual Cycles. The upper plot of figure 2 depicts the day to day changes in potential between each pair of fingers of a subject (case 66) with normal menstrual cycles. There is no evidence of any cyclic variation with reference to the onset of catamenia, nor do these data demonstrate any unique property possessed by the index fingers (no. 2). In general, our findings confirm the statement of Parmenter (16) that there is "no consistent unidirectional polarity of the right index finger."

The lower plot of figure 2 represents the finger potentials of a normal menopausal individual (case 87). There is no significant difference between this graph and the upper one. Furthermore, the presence of minor excursions in the plot from the menopausal subject serves to indicate that similar fluctuations recorded for no. 66 are not pertinent to menstruation.

Likewise, data from one pregnant subject, one surgical castrate, and two women who doubtless had anovulatory cycles, did not differ from records of those with normal menstrual cycles.

No significant differences in the finger potential curves of a cyclic nature were observed between individuals. Some subjects tended to show a greater day to day variation than others, but this remained a constant factor. As in the case of the isopotential contour determinations reported above, the individuals who exhibited the greater day to day variations of potential were those of the "sthenic" type.

[5] In the experiments on finger potentials reported by others (14, 15, 17, 18), the Burr-Lane-Nims vacuum tube voltmeter was used (21). Parmenter (16) also employed a meter-indicating type of vacuum tube voltmeter.

From a consideration of these individual curves, we have been completely unable to confirm the observations of other workers who have reported a significant rise of potential between the index fingers associated with ovulation.

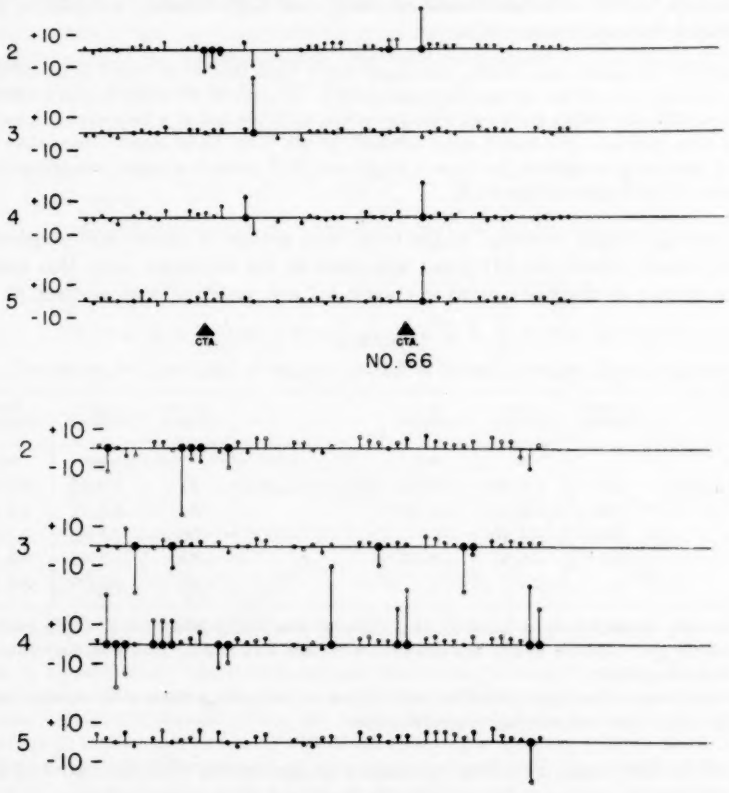


Fig. 2. *Upper plot.* Daily finger potentials, plotted in millivolts, of a normal, healthy female (no. 66). The left finger of the pair being measured was arbitrarily used as the reference finger. The potentials are plotted for each pair of fingers except the thumbs (# 1 fingers). It was not practical to use the thumbs due to the prevalence of cuts, bruises, etc. The solid black circles on the time axis indicate when the potential measured was considered invalid due to skin damage, such as cuts and hangnails. The solid black triangles represent the beginning of the menses. It is quite apparent that the index fingers (# 2) are not unique in so far as potential changes are concerned.

Lower plot. Daily finger potentials of a normal menopausal subject (no. 87). Data obtained concurrently, with the same equipment and in the same way as those shown in the upper graph. The solid black circles here also represent potentials as measured, but considered invalid due to skin damage. There is no significant difference between the two plots shown in this figure. The greater number of invalid potentials recorded in the lower graph is probably due to the fact that subject 87 performed considerably more manual work than subject 66. The hands and fingers of the former were injured very frequently.

Magnitude and Polarity of Potential Changes. When great care was taken to check spurious finger potential measurements, no voltage reading higher than 12 mv. was found to be valid. Moreover, only three or four out of approximately 14,000 determinations showed this high figure. Usually 7 mv. represented the maximum value.

All potentials higher than 12 mv., and many lower than this, were found to be spurious because of cuts, pin-pricks, hangnails, bruises, etc. It must be strongly emphasized that it is practically impossible to detect visually—even with the aid of a magnifying lens—all types of skin damage. We found ethyl alcohol (95 per cent) to be more dependable as a means of detecting abrasions, for even a slight one will cause a stinging sensation upon immersion of the fingers in this fluid.

The average finger potential of the combined groups of nurses and employees was +1.7 mv. Since the left hand was used as the reference side, this means that the fingers of the right hand averaged 1.7 mv. positive with respect to the

TABLE 1

Average monthly finger potentials of female subjects in relation to "handedness"

	SUBJECT NUMBER	APRIL AVERAGE	MAY AVERAGE		SUBJECT NUMBER	APRIL AVERAGE	MAY AVERAGE
		mv.	*mv.*			*mv.*	*mv.*
Left-handed	67*	−1.20	−0.68	Right-handed	51	+2.11	+1.40
	78†	−0.31	−0.29		54	+3.71	+5.00
	80	−4.16			60	+4.40	+4.13
	102	−0.81	−0.57		63	+3.11	+3.89
					67	−1.20	−0.68

* Although normally right-handed, this subject was doing filing work which necessitated pulling and pushing heavy file drawers with her left hand. She was therefore included in both groups.

† This subject, whose finger potential was very near zero, was a nurse who assisted in the operating-room and was notably ambidextrous.

fingers of the left hand, a finding in reasonable agreement with the figure of 2.25 mv. published by Barton (17) for index fingers, and of the same polarity.

Relation of Polarity and "Handedness." However, four left-handed subjects in the group all averaged negative instead of positive; that is, the right hand was negative to the left one. The finding of a polarity which seems to be related to the "handedness" of the individual parallels in electric terms the temperature observations of Heiser and Cohen (22) who reported that in four out of five right-handed subjects tested by them, the right wrist was warmer than the left one, while in a left-handed person, the converse was true.[6] In attempting to account for this difference, these authors suggested that it might be due to the "relative dominance of the limbs."

Table 1 shows the difference in polarity of finger potentials between three left-handed and five right-handed individuals. Subject 67 appeared at

[6] One of us (J. M. S.) had the opportunity of examining more extensive data of Heiser and Cohen which confirmed these findings but were not included in the original report cited.

first to be an exception; that is, although right-handed, she steadily maintained a negative charge. The apparent discrepancy here was later explained by the fact that this woman's duties were largely concerned with pulling and pushing heavy file drawers with the *left* hand. She is therefore included in both groups.

It is impossible for us to explain the constancy of opposite polarity in finger potentials related to handedness if the electric quality of the finger-tips is assumed to be referable to the ovaries.

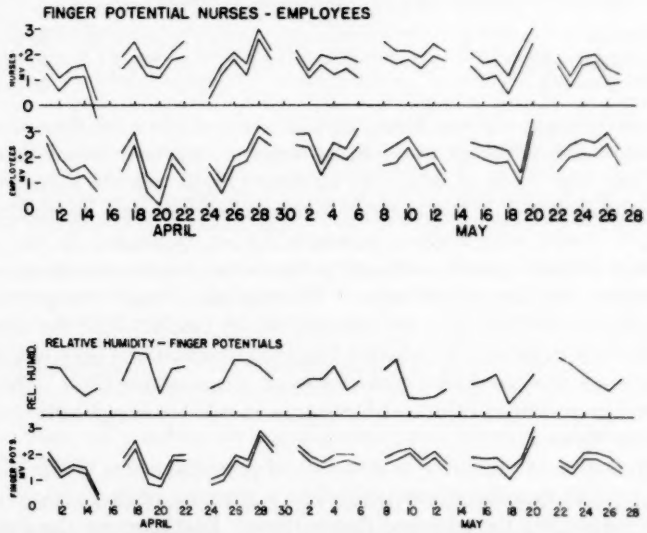


Fig. 3. *Upper curves.* Daily average finger potentials of the nurses and female employees plotted as separate groups. Bands for each group indicate limits of probable potential averages. Potential changes within the bands are not statistically significant, and the curves are in reality indeterminate within the zone. The similarity of the band changes indicates some factor common to both groups which modifies the magnitude of the finger potentials. The sign of the finger potentials obtained from the left-handed subjects listed in table 1 has been changed to a positive value in plotting these curves.

Lower curves. Total combined average daily finger potentials of nurses and employees plotted against the relative humidity. The finger potential curves are subject to the same restrictions as outlined for the upper curves of this figure. Since there are now more observations for each day, the band width is appreciably less.

The Possible Rôle of Environmental Factors in Voltage Changes. When the mean daily finger potentials of all individuals within each of the two groups (group 1, nurses; and group 2, employees) were averaged, and the average of each group was plotted separately, the similarity of the two graphs during some weeks is quite evident (fig. 3, upper curves). The two bands plotted for each group show the maximum and minimum probable variations in potential for a given day. The average potential falls between the two bands, and even though it may be calculated and assigned a mathematical quantity, it is in reality statistically indeterminate within the bands.

Considering these limitations, we find that the potential change of both groups from day to day was in the same direction 86 per cent of the time. Such a relation indicates some common environmental influence acting on both groups. In order to check this hypothesis, several environmental factors were considered: namely, temperature, barometric pressure and relative humidity. Daily measurements taken from the noon observations of the Boston Weather Bureau were utilized. They showed the following percentages of changes in the same direction as the combined group potential averages:

Temperature	52 per cent
Barometric pressure	55 per cent
Relative humidity	66 per cent

These percentages are not large, and in view of the fact that the various subjects worked in different parts of the hospital, the only factor listed which possessed any high order of similarity between groups was the barometric pressure. Obviously neither the outdoor temperature nor any particular indoor temperature would constitute a common factor. Changes in the external relative humidity are usually reflected in the indoor relative humidity, although not necessarily in a one-to-one ratio. The changes in finger temperature with variations in relative humidity are complicated by the fact that the direction of the temperature shifts due to relative humidity fluctuations may change phase depending upon the particular environmental temperature (23). The correlation of average potential values with changes in relative humidity is seen in the lower plot of figure 3.

The high degree of similarity in direction of potential shifts in the two groups of women studied simultaneously pointed to a common environmental influence as a factor underlying the observed fluctuations. Furthermore, the observation that the more active, and possibly, warmer, hand appeared to show a positive polarity with respect to the less active one, suggested the investigation of temperature as a factor in finger-to-finger potentials.

III. The thermal factor in "finger-to-finger potentials." An experiment was therefore devised whereby the electric potential difference between corresponding fingers of opposite hands (or fingers of the same hand) could be measured while the temperature of the saline solution that served as electrode for one finger was varied slowly (about 1°C. per min.) between the limits of 12° and 46°C.[7]

Procedure. Two one-liter pyrex beakers were filled with physiologic saline solution. The fluid in one beaker was at room temperature, and in the other at some temperature below 10°C. Each beaker was connected by a salt bridge to a Ag-AgCl electrode system in saline solution at room temperature. The Ag-AgCl electrodes in turn were connected to a special Leeds & Northrup Multi-point Micromax Recorder (L. & N. Catalog no. 40356–S–4). This recorder was converted so that a continuous tracing of the potential was made. It was designed to operate over a very wide range of input resistances. Satisfactory records could be obtained from circuits which varied from zero to 250,000 ohms. The chart paper was moved at a uniform rate by means of a synchronous motor-drive system.

[7] It may be well to point out that finger temperature is not necessarily the same as the environmental temperature, but may easily differ by 10°C. from the latter (23, 24).

The beaker containing the cold salt solution was placed on a 500-watt electric heater. Electric energy was supplied to the heater to increase the temperature of the saline solution at a uniform rate of 1°C. per minute. Since the amount of heat needed changed constantly throughout the experiment, automatic control was required. This was provided by a Leeds & Northrup Model N control instrument. The controller was activated by means of a resistance thermometer placed in the cold salt solution. The controller contacts were capable of carrying the electric heater current without auxiliary relays. The controller was designed to regulate temperature over a range of 0° to 50°C. In order to raise the temperature of the solution at the rate of 1°C. per minute, a General Electric Telechron motor drive was coupled rigidly to the shaft of the control point-setting dial of the L. & N. controller. The synchronous Telechron motor then turned this dial at a rate which increased the temperature of the saline solution 1°C. per minute. The actual error due to thermal lags, etc., was not more than ±0.1°C. at any instant. In practice, the temperature of the cold beaker was initially less than 10°C. This was necessary so that the controller might have time to overcome the large thermal lag of the cold heating system. With this precaution in starting, the rate of temperature increase was constant slightly before 12°C.

When the temperature of the saline solution reached 11°C., the subject dipped the index finger[8] of the left hand in the beaker of saline solution at room temperature, and the third, fourth and fifth fingers of the same hand, in the cold salt solution. The electrode circuit was in contact with the recorder between 12° and 46°C.

The recordings in the present investigation differ from those obtained in the daily finger-to-finger measurements reported above. In the experiments described here, the beaker of solution to be heated was used as the reference electrode. This was done merely for the sake of convenience, so that the curves, as recorded, read correctly from left to right, and the potential is represented on the chart as increasing in an upward direction.

The fluid in both beakers was automatically stirred vigorously throughout the experiment. Since the chart speed was constant, and the rate of temperature increase was likewise a constant, the resulting curve was automatically traced in the proper rectilinear coördinate system.

Curves Relating Thermal to Potential Changes.[9] Using this procedure, over 200 thermal potential difference (T. P. D.) curves have been recorded. Approximately 100 of these, obtained from daily measurements on the same subject throughout four normal menstrual cycles, show homogeneity within a given cycle, although the character of the curves may change from cycle to cycle. The recordings during the first and fourth cycles appear quite similar. Occasionally the repetition of detail in a curve from day to day is striking. Daily recordings usually show the changes to be progressive, but they may at times be abrupt. Figure 4 illustrates several different types of curves that may be obtained.

The magnitude of potential change with temperature averages approximately 1 mv. per degree Centigrade. However, considerable departure from this average may be observed.

When the temperature was kept relatively constant, as is illustrated in curve *A* of figure 5, the potential after an interval of 5 minutes maintained a fairly uniform level. The slight slope of the potential curve after the first 5 minutes

[8] The reason for using the index finger was simply for convenience; any other finger gives the same results.

[9] The thermal potential difference data have been independently checked at Loomis Laboratory by Dr. Hallowell Davis of the Department of Physiology, Harvard Medical School (25).

was doubtless due to the fact that one of the beakers of saline solution unavoidably warmed up from 24.1° to 25°C. Curve *B* was obtained immediately after curve *A* and shows the finger potential change over the range of 12° to 46°C. It is interesting that when the temperature reached 24.4°C., the voltage recorded on curve *B* was equal to that on the isothermal curve *A* at the same temperature.

It should be noted that position did not affect the T. P. D. curves; that is, the results were the same, whether the subject was sitting up or resting horizontally on a comfortable bed. A cut on the finger invariably reduced the magnitude

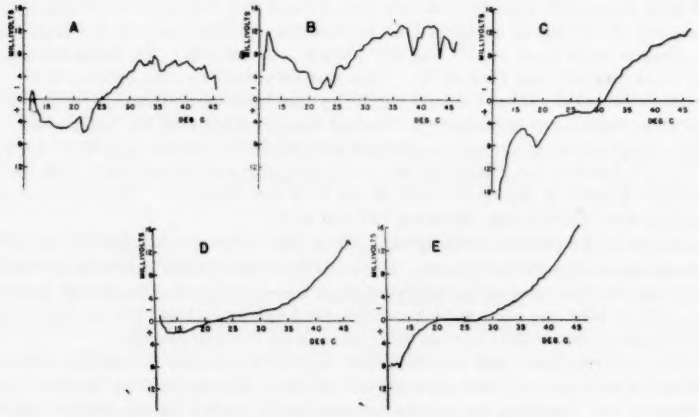


Fig. 4. Thermal potential difference curves illustrating the variety of these. These have been plotted from data recorded by the Leeds & Northrup Micromax instrument before the temperature axis was made uniform. Other T. P. D. curves mentioned in the text, but not shown for lack of space, were plotted automatically on a linear temperature axis by the method described.

Curves *A*, *B*, and *C*, from the young woman who was studied for four normal cycles, were taken at intervals of several days.

Curve *D* was obtained from a young pregnant woman who gave this same type of curve repeatedly.

Curve *E*, recorded from an anovulatory female, represents the general type most frequently obtained from such women and from male subjects.

Curves *D* and *E* should not be regarded as characteristic of the individuals. They are merely those most frequently encountered in the subjects mentioned. Exceptions have been recorded, and as yet there are not enough data to warrant specific conclusions.

of the potential change and at the same time usually caused a large shift in the base-line. The cut also tended to "short circuit" the normal T. P. D. effect.

Thermal Potential Curves During Narcosis. In order to rule out any possible effect on the subject of extraneous stimuli which might influence the nature of the potential changes observed, a curve was recorded 1½ hours after the administration of 3 grains of pentobarbital. The subject was completely asleep and entirely unaware of the experiment in progress. There was relatively little difference between this curve and one obtained with the subject wide awake. Likewise, a recording made while she was under deep ether anesthesia did not differ essentially from curves obtained while she was awake. Therefore, what-

ever the origin of these potentials, it is evident that they are not greatly modified by narcosis or anesthesia.

Reversibility of Thermal Potential Changes. In an effort to ascertain whether or not the mechanism giving rise to these potential differences constitutes a reversible process, the usual curve was recorded as the temperature rose to 46°C., but when that point was reached, the temperature was lowered at the same rate (1°C. per minute) to 12°C. The reversible nature of the thermal potential changes, clearly demonstrated in the right-hand curves of figure 5, is particularly interesting from the standpoint of thermodynamics.

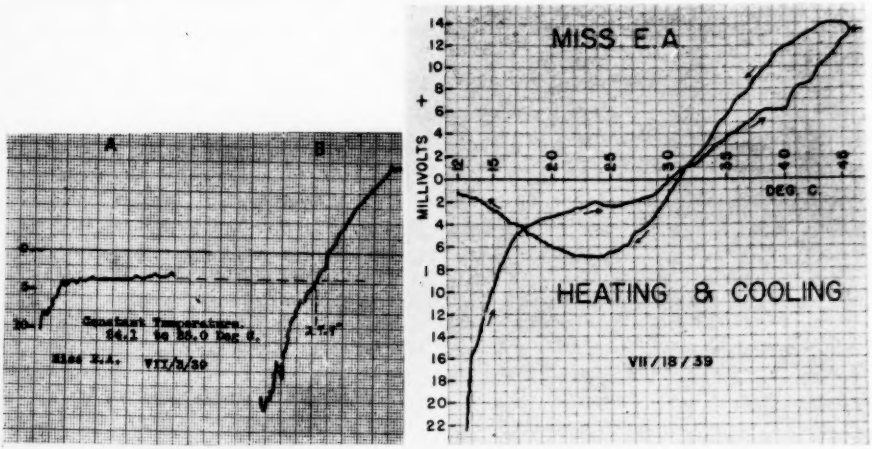


Fig. 5. *Left-hand curves.* Curve *A* shows the potential obtained under approximately isothermal conditions. Potentials are in millivolts. The potential changes comparatively rapidly during the first 5 minutes and then changes very slowly.

Curve *B*, taken immediately after curve *A*, was recorded in the usual way with the temperature increasing 1°C. per minute from 12° to 46°C. Note that the potential at 24.4°C. is the same in both curves.

Right-hand curves. Thermal potential difference curves showing reversibility of process by heating and cooling. The saline solution was warmed at the rate of 1°C. per minute up to 46°C. and then cooled at the rate of 1°C. per minute to 12°C. The arrows indicate the direction of the potential change. The difference between the curves below 23°C. may or may not be significant. Some individuals show a fair degree of similarity between the two curves and others do not, as in this case. Curves plotted from data obtained by the L. & N. Micromax Recorder.

The Sympathetic System and Thermal Potential Changes. It is reasonable to suppose that the general shape of the T. P. D. curves, in regard to both gross and minor features, may be due to nervous control of the vascular system. To check this possibility, curves were recorded from a control and from a sympathectomized rabbit, measurements being made between the ears of each animal.[10]

[10] Dr. Philip M. LeCompte and Dr. Hallowell Davis of the Department of Physiology, Harvard Medical School, kindly assisted in the rabbit experiments. Doctor LeCompte carefully sympathectomized the rabbits several days preceding the experiment.

Sympathectomy, performed about six days prior to the experiment, consisted of removal of the superior cervical ganglion and section of the right dorsal and ventral auricular nerves at the base of the ear. The rabbit to be tested, narcotized by an intraperitoneal injection of pentobarbital, was fastened on its back on an animal board, with the head placed so that the ears hung down over the end of the board and into the beakers containing the saline solution. The right ear was immersed to a depth of approximately 7 cm. in the beaker to be heated, and the left ear was similarly dipped in the solution maintained at room temperature.

The change of potential recorded in these curves was about 0.5 mv. per degree Centigrade. The shape of the curve is clearly unaffected by any function of the sympathetic nervous system. This experiment was repeated on the same control rabbit and on a second sympathectomized animal with practically identical results.

The curves obtained from rabbits seem to possess no features which might distinguish them from curves of human subjects.

IV. THE RÔLE OF TEMPERATURE IN ABDOMINOVAGINAL POTENTIALS. In view of the evident influence of temperature on finger-to-finger potentials, it was of interest to ascertain whether or not the same general relations would hold true in abdominovaginal voltage determinations.

Isothermal Contour Patterns. Since the reference electrode employed in measurements of the so-called "abdominovaginal sign" of ovulation had been applied on the suprapubic skin region (1–3, 6, 9–12), it seemed necessary, first of all, to check the degree of temperature variations over skin surfaces. This was accordingly performed over the abdomen and thighs of a human subject. The results are conveniently represented by isothermal contours (fig. 6).

Procedure. The temperature was measured by means of a small thermocouple of no. 42 B. & S. gauge copper and constantan wire. The exploring thermal junction was mounted on the surface of a piece of cork 2 mm. square, which in turn was fastened to a small glass tube. The exploring junction had a very small thermal capacity and reached equilibrium with the skin in a fraction of a second.

The reference junction was immersed in a water bath at approximately room temperature inside a vacuum Thermos flask. This gave sufficient stability to the temperature of the reference junction, since there was at most a differential of one or two degrees between the reference junction water bath and room temperature.

The thermocouple system was calibrated, each time it was used, by placing the exploring junction in a water bath containing a standard mercury thermometer graduated in steps of 0.02°C. The water bath was then warmed over the range of skin temperature previously measured. A Leeds & Northrup (Catalog no. 2500-C) Type R galvanometer was used as a null-indicating instrument.[11] Even with the relatively long natural period of 5 seconds, it was easy to obtain sufficiently accurate temperature determinations in 3 to 4 seconds. The short time is made possible by anticipating the deflections of the galvanometer and rapidly manipulating the potentiometer to keep the deflection at the zero point.

Results. The isothermal contour patterns (fig. 6) closely resemble the isopotential curves, as recorded in figure 1, and show clearly how the temperature

[11] This was not the proper galvanometer for the purpose, but was the best available at the time.

varies over the particular surface areas investigated. By checking the contour lines it is readily seen that points only 4 or 5 cm. apart may differ in temperature as much as 2° to 3°C. As regards stability, the isothermal and isopotential patterns are similar.

Benedict, Miles and Johnson (26) have published temperature curves obtained by moving a thermocouple down the nipple line of a human subject. Similar curves may be derived from figure 6 by plotting the temperature values along a straight edge placed parallel to the nipple line.

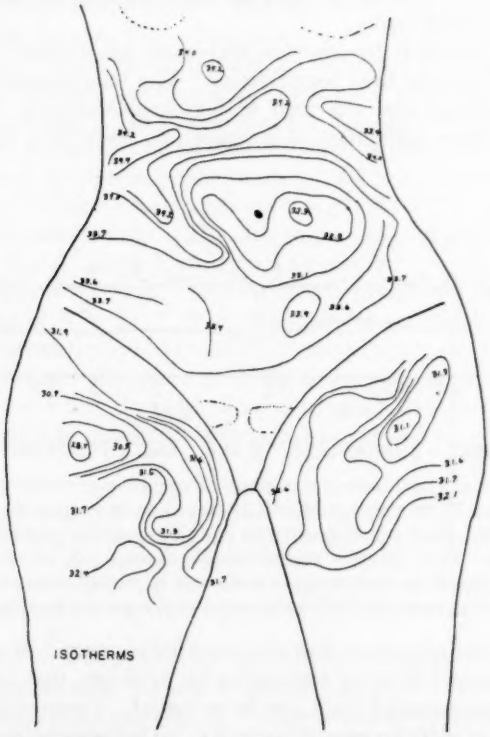


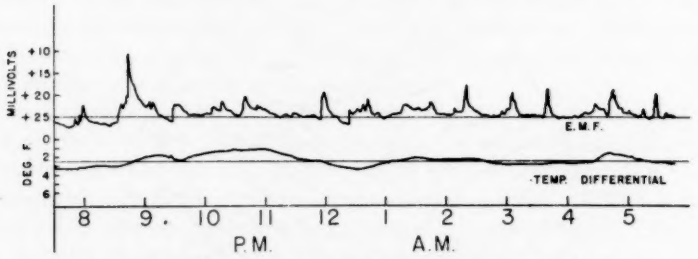
Fig. 6. Isothermal contours measured on a female subject. The isothermal contours exhibit the same type of "highs" and "lows" as do the isopotential patterns; cf. figure 1. The temperature range of the isotherms shown in figure 6 is from a "low" of 28.4° to a "high" of 34.4°C., a difference of 6.0°C.

Thermal and Potential Abdominovaginal Gradients. Figure 7 is a traced composite of a portion of the electric and thermal record obtained simultaneously when a vaginal and a suprapubic electrode containing thermocouples were employed. Such an experiment is interesting in view of the report of Burr and Barton (27) in so far as it furnishes data on the variation of another parameter: namely, temperature, which is known to fluctuate during sleep.

Procedure. The electrodes were agar blocks made with physiologic saline solution, agar and Arlex.[12] The electrode proper consisted of a chlorided silver plate immersed in the agar mass before the latter solidified. Small iron-constantan thermocouples inserted in thin-walled glass capillary tubes were also embedded in the agar mass. The thermocouples were connected so that each agar block electrode contained two thermal junctions to increase the temperature sensitivity.

The output of the thermocouples was connected to a Leeds & Northrup Micromax Recorder. The potential leads were connected to another Leeds & Northrup Recorder. The L. & N. Recorders had the same paper speeds and each was operated by a synchronous motor.

Results. Unfortunately the agar electrode mass has a relatively large thermal capacity, as well as poor heat conductivity. The temperature record is therefore greatly smoothed and will tend to lag considerably in time. However, even in spite of these difficulties, it is reasonably clear from figure 7 that the



VAGINAL—SUPRAPUBIC E.M.F. AND TEMPERATURE

Fig. 7. Composite of simultaneous recordings of vaginal-suprapubic potential and temperature *differences*. These were automatically traced on two separate Leeds & Northrup Micromax Recorders. Each record was traced and the composite carefully put together to insure simultaneity. There is quite an appreciable thermal lag, as recorded, due to the poor heat conductivity of the agar mass, as well as its relatively large heat capacity. The temperature record may be seen to follow the base-line of a greatly smoothed electric record.

mean changes in temperature follow the potential changes. Most of this record shows the electric and thermal tracings to be in phase; that is, an increase in temperature is accompanied by a rise in potential. Frequently, however, the phase relations may shift for several hours; i.e., an increase in temperature now is accompanied by a *decrease* in potential. These potential-temperature phase reversals are entirely consistent with the results obtained in the thermal potential difference experiments. As shown in curve *A* of figure 4, the potential may not increase uniformly over the temperature range of 28° to 34°C., the same temperature range as represented in the isothermal plot of figure 6. In curve *A* of figure 4, the potential is seen to increase with temperature from 28° to 31.8°C., and then it proceeds to fall 1 mv. between 31.8° and 32.5°C., even though the temperature is still rising.

[12] Arlex: Trade name of commercial sorbitol, an excellent "humectant", manufactured by the Atlas Powder Company, Wilmington, Delaware.

It should be emphasized that both the thermal and electric curves of figure 7 represent differential values; that is, the temperature or electric *difference* between the vaginal and suprapubic electrodes.

V. pH AND POTENTIAL CHANGES. Since the experiments described above clearly indicated a relation between temperature and bioelectric potentials, the next consideration was the effective mechanism.

Evidence that the thermal factor might act through its influence on pH was suggested by the data of Sribyatta and Bazett (28) on decrease of pH of blood in vivo with increase in temperature. Through a temperature range (10°–45°C.) approximately the same as that within which the T. P. D. curves of the present study were recorded, these workers obtained a difference of 0.36 pH. Now, if one assumes that the thermal skin temperatures are due to a pH concentration-cell system made up of blood, tissue fluids and the skin, and since a change of 1 pH unit is equal to a potential shift of about 60 mv., a decrease of 0.36 pH would involve an increase of approximately 22 mv. This figure is of the same order of magnitude as the voltage changes recorded in the T. P. D. curves over a corresponding temperature range. The pH values, as plotted by Sribyatta and Bazett, and the T. P. D. curve C of figure 4 are similar.

Stasis and Potential Changes. In view of this agreement between calculated and observed values of E. M. F. when thermal skin potentials were assumed to be due to a pH concentration-cell system, we next undertook to determine if one could obtain significant changes in potential by employing some means to affect pH. An experiment was therefore devised whereby the temperature was kept constant, but a shift in blood and tissue pH was induced by arterial and venous stasis.

The same set-up was used as previously in obtaining the T. P. D. curves represented in figure 4, but in this case the fingers of the experimental hand were placed in a beaker of saline solution kept at a constant temperature of 40°C. ±0.1°C., while the reference or contralateral fingers were immersed in salt solution maintained at approximately 23.2°C. A Riva-Rocci cuff was placed on the experimental arm as for determinations of blood pressure.

For a period of 20 minutes normal conditions prevailed. Then the cuff was rapidly inflated to 40 mm. Hg above systolic pressure. Following a brief lag, the potential rose to a plateau (fig. 8). After 10 minutes, during which pain was experienced and the arm became quite cyanotic, "Bier" spots being clearly visible near the end of this period, the pressure in the cuff was rapidly released, whereupon the potential fell steeply. There was, however, a lag of 10 to 15 seconds between the release of the pressure and the beginning of the potential drop. The potential decreased rapidly and seemed to overshoot—possibly because of excess blood flow due to the relaxed condition of the capillaries under the high concentration of CO_2 which had been built up during the period of arterial and venous stasis.

When the cuff was again inflated 10 minutes later, the same phenomenon was observed. In the last part of the record, a similar potential rise was induced by warming the experimental hand.

This particular experiment may be repeated as often as desired with substantially identical results.

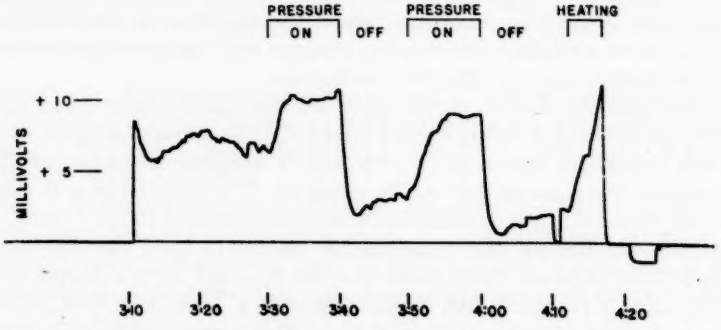


Fig. 8. Finger-to-finger potential changes induced by arterial and venous stasis by means of a Riva-Rocci cuff on one arm.

When the pressure was released at 3:40 p.m., there was a sudden drop of 8.8 mv. in the potential difference between the control and the experimental arm. From data compiled by Peters and Van Slyke (29), a difference of approximately 0.14 pH may be expected in human blood between the condition of rest and that of heavy exertion. This corresponds to a voltage shift of 8.7 mv., a figure in close agreement with our result.

The last part of the record marked "heating", and beginning at about 4:11 p.m., shows the potential change on warming from 40° to 46°C. The break in the curve is caused by the momentary cessation of the heating at 43°C.

Curve reproduced photographically from untraced original.

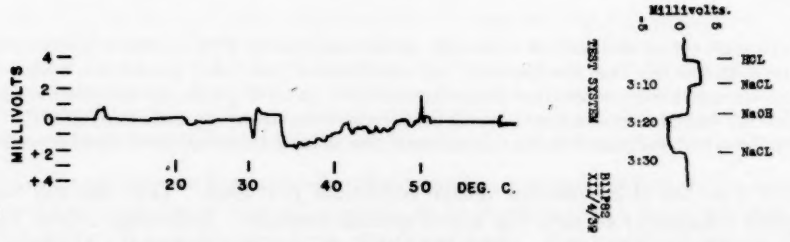


Fig. 9. *Left-hand curve.* Thermal potential differences induced by heating heparinized whole blood in one arm of a "U"-tube, the ends of which were covered with a semipermeable membrane. Little potential change appeared before 30°C. At 50°C. the potential fell to zero and no further potential change was observed, even though the heating was continued to 60°C. It is quite possible that the 50°C. point may be significant in this connection, as most of the proteins would be completely denatured at or before this temperature was reached. The curve is an untraced photographic reproduction.

Right-hand curve. Potentials recorded in test system when dilute HCl and dilute NaOH are added on one side of the semipermeable membrane. Curve traced from original recording.

VI. Effects of temperature and pH potential in vitro. In order to study directly the effect of thermal and pH changes on potential, experiments were performed on a synthetic system.

1. *Temperature.* The set-up consisted of a "U"-tube containing heparinized whole venous blood; across the ends of the "U"-tube was stretched a semipermeable membrane. One arm of the "U"-tube was inserted in the beaker of saline solution serving as the reference terminal. The other arm of the "U"-tube was dipped in the salt solution to be heated. The curve obtained, as the saline solution was heated, is shown in figure 9 (left-hand plot).

2. *pH.* The "U"-tube used above was modified by making a glass "T" connection at the vertex of the inverted "U" and inserting a very small bore rubber tube down the "T" and along one arm of the "U". The side having the rubber tube was called, for convenience, the "experimental arm." In the set-up as described above, but with the "U"-tube filled with the same saline solution as in the beakers, the only potential in the system was the small electrode potential.

When dilute HCl in saline solution was run through the rubber tube to the "experimental arm," a potential change in the positive direction was observed, as shown in the right-hand curve of figure 9. When pure saline solution was substituted, the potential returned to the initial value. The introduction of dilute NaOH in salt solution, on the other hand, was followed by voltage changes in the opposite direction.

The direction of the potential change elicited upon introducing dilute HCl into the system was the same as that caused by the acidosis resulting from the arterial and venous stasis produced in the experiment described above. The results of the in vitro experiments therefore substantiate those obtained in vivo, showing clearly a definite relation between thermal and pH factors and potential.

Discussion. The evidence presented in figure 1 indicates the great difficulty of locating on the skin surface a stable reference electrode. The potential gradient is occasionally so steep that a difference of 22 mv., or more, may be found between two points no further apart than 4 cm. This difficulty in maintaining the stability of a reference electrode on the skin surface offers in itself a rational explanation for the inconsistent results previously obtained in connection with the so-called "abdominovaginal sign" of ovulation.

The rôle of emotional factors in relation to stability of isopotential contour patterns brings up an interesting point with regard to the findings of Altmann (8) on the sow. Since Altmann's potential values, as published, represent the averages of several daily observations taken at various times during the cycle, it is not surprising that the voltage differences at ovulation time are higher than those at diestrus, for she herself comments on the "frequent and typical grunting, mounting, and restlessness" of the sow during heat. She records that in the course of estrus, spontaneous activity increased up to about twice the non-estrous level.

Inasmuch as in our experiments on finger-to-finger potentials we were unable to duplicate the results of other workers who reported cyclic electric changes which they correlated with ovulation time, it may be pertinent to remark on certain aspects of these studies. In regard to the findings recorded by Barton (17, 18), it should be pointed out that the method of reducing all cycles to deciles grossly ignores any possible biologic connection between ovulation and menstruation. While the method is admittedly statistically convenient, it may not legitimately be applied where there exists any causal relation between a series of variables observed over different intervals of time, and especially when there is a functional dependent relation between the separate observations.

In connection with the experiments of Altmann et al. (19), it should be noted that since the cycle lengths of their subjects ranged from 23 to 61 days, the results are hardly applicable to the normal cycle. Furthermore, the temperature and vaginal smear data, collected for the purpose of furnishing correlative and supporting evidence as to the time of ovulation, were admitted by the authors to be not entirely satisfactory. The temperature records, they state, did not always represent basal values. The vaginal smears, stained by the original technic of Papanicolaou, which is now considered quite inadequate, manifested a "rather weak expression of the phases of the cycle." The latter was attributed to the fact that the subjects were college women.

The temperature potential difference (T. P. D.) curves recorded in this study clearly demonstrate a definite relation between thermal and electric changes, and indicate that the mechanism by which temperature influences potentials is concerned, at least in part, with changes in pH. Certain non-linear results obtained in the course of our experiments bring to mind the investigations of Loeb (30) on the effect of hydrogen-ion concentration of protein solutions in modifying the potentials across membranes.

On the basis of the asymmetric temperature differences noted by Heiser and Cohen (22), as well as the finger potential data presented above, it would seem highly probable that there is a small but significant pH difference between the venous blood taken from the right and left arms. In right-handed individuals, the pH of the blood should theoretically be slightly lower in the right arm than in the left one.

It may be pointed out that changes of blood and tissue pH with respect to temperature have been little investigated. Measurements of pH have usually been made under carefully standardized temperature conditions, and comparatively meager information is available relating to continuous functions of temperature and pH. The influence of environmental factors on blood pH was investigated by Berg and his associates (31) who found a high statistical correlation between the blood pH of dogs and that of human beings studied over the same period of time. The relation between pH and temperature was less marked than that between pH and barometric pressure. In our own study on finger potentials made simultaneously on two groups of women, very little correlation could be shown between the observed potentials and barometric pressure, whereas a definite relation was noted between potentials and relative humidity. This is probably due to the fact that the action of the pressure change is symmetrical and does not have as large an asymmetric modifying factor as does the relative humidity. The relative humidity may be considered as an asymmetric modifying factor, since there exists an initial difference in temperature between the right and left hands and any change in the relative humidity modifies the relative rates of heat radiation of the two hands.

In attempting to explain the marked E. M. F. changes that have been associated with ovulation, one should take into account the experiments of Mittelmann and Wolff (24) who reported great variability in finger temperatures with different emotional states. *Since skin temperature fluctuations are known to be induced by vasomotor and hormonal changes, it seems quite probable that the so-called "ovulation potentials" previously reported, whether elicited between a vaginal and a suprapubic electrode (1–6, 9–12), between two surface areas—abdominal or flank (7, 8, 12), or between fingers (14–19), are measurements only of local changes in*

peripheral cutaneous blood flow, due to alterations in capillary tone. Hormonal changes, which would of necessity be general and diffuse, might easily lead to increased instability of capillary tone, giving rise to fairly large thermal and potential shifts. On this basis, one may perhaps explain the difficulty encountered in attempting to plot either isopotential or isothermal contours on individuals of the so-called "sthenic" type. When one considers the profound vascular and thermal changes which occur with estrus, Rogers' (6) experiments on rats seem to fall readily in line with the results in human beings and rabbits.

In regard to the contention of Altmann et al. (19) that potential and temperature differences between index finger-tips were unrelated, it should be pointed out that no definite conclusion can be drawn from their experiments, for, whereas the thermocouple measures the temperature of a decidedly restricted area in physical contact with its elements, the electric measurements, on the other hand, represent the bioelectric conditions existing over a fairly large area (the surface of the finger-tip), and would not be expected to be related to a point determination of temperature.

Mention should here be made of a recent report by Langman and Burr (32) of a negative shift in potential difference between the cervix *uteri* and the ankle noted at certain times in the menstrual cycle, whereas tests made on other days of the cycle showed the cervix to be positive to the ankle. Negativity was observed during the menses, as well as at various times in the intermenstruum. Because in two cases artificial insemination performed during the negative phase resulted in pregnancies, the authors regard the negative shift of potential of the cervix *uteri* as a sign of ovulation.

In this connection it seems pertinent to suggest the possibility that the potential change recorded by Langman and Burr may be dependent on cyclic variations in the pH of the cervical mucus. Pierra (33), reporting an increase in alkalinity of cervical mucus in the intermenstruum, stated that while the rise in pH could not be considered a definite test of ovulation, it might help to determine when a woman was especially fertile. Later, Lamar, Shettles and Delfs (34) noted increased alkalinity of cervical fluid during the menses, as well as in midcycle, and observed that this change was accompanied by a rise in the amount of secretion together with increased penetrability to spermatozoa, as well as longer survival time of the latter. The possible rôle of cervical pH in accounting for unexplained causes of sterility has been suggested by Schockaert and Delrue (35).

May not the increased negativity of the cervix during catamenia and in the intermenstruum, recorded by Langman and Burr, be merely a measure of increased alkalinity of cervical secretion at these times? Such a process can be duplicated by the in vitro experiment described above (fig. 9, right-hand curve) in which a shift of potential difference in the negative direction took place when NaOH was substituted for NaCl in the synthetic system. On this basis, then, one would expect the negative phase to be the logical time for artificial insemination to succeed, due to the increase of alkalinity of the cervical mucus associated with optimum conditions for penetration and longevity of spermatozoa. Strictly speaking, then, the negative shift in potential between the cervix *uteri* and the ankle, reported by Langman and Burr, may be regarded not as a test for

ovulation, but as an indication that conditions in the cervix are favorable for the survival and successful functioning of spermatozoa.

SUMMARY AND CONCLUSIONS

1. By plotting isopotential contour patterns on human skin areas, it was difficult to locate properly on the surface a reference electrode which would be stable in potential. This instability, which may be in part, at least, related to emotional factors, made it impossible in our cases to obtain isopotential contours on preoperative patients near ovulation time. With the broad ligament as a reference electrode, direct measurements on ovaries *in situ*, during laparotomy within the estimated ovulation time, failed to show any marked potential gradients. The difficulty of maintaining the stability of a reference electrode on the skin surface offers in itself a rational explanation for the inconsistent results previously obtained in connection with the so-called "abdominovaginal sign" of ovulation.

2. When adequate precautions were taken to rule out spurious readings, no marked changes could be demonstrated in daily finger-to-finger potentials of two groups of female subjects studied for about nine consecutive weeks. In women with normal menstrual habits, there was no evidence of cyclic potential changes between finger-tips that might be related to the occurrence of ovulation. The index fingers did not appear to possess any unique property when voltage readings obtained from them were compared with determinations made on other pairs of fingers. The observation that polarity was associated with relative activity (and possibly temperature) of one hand as against the other, as well as the high degree of similarity in direction of potential shifts of the two groups of women studied simultaneously, pointed to a common environmental influence as a factor underlying the observed fluctuations, and suggested the investigation of temperature in relation to finger potentials.

3. Temperature was shown to influence bioelectric potentials, the effect consisting, as a rule, of an increase in potential of the warmer area in a positive direction with respect to the cooler one. The relation of skin potential to the surface temperature was found to be reasonably stable and reproducible within certain limits over the range from 12° to 46°C. The change of potential with temperature was reversible; it was not affected by the sex, state of consciousness, or position of the subject. The results were similar whether fingers of the same or of opposite hands were tested, and, as shown by the rabbit experiments, were apparently not dependent on sympathetic innervation of the areas studied.

4. The evidence presented suggests that the mechanism through which temperature affects potential may be bound up with changes in pH. If one assumes the thermal skin potentials to be due to a pH concentration-cell system and, on the basis of previous experiments relating temperature and pH, one calculates the values of E. M. F., the theoretical and observed figures agree closely.

5. Bioelectric phenomena previously reported as related to ovulation are due primarily to cutaneous vascular conditions and possibly only remotely and to a

slight degree to ovarian influence. Local changes in blood flow affect the focal temperature, which in turn reacts on the focal pH, the fluctuations of which directly determine potential variations. Because the hormonal factor is only one of several affecting vascular phenomena, it is our belief that potential changes recorded thus far cannot be attributed solely to ovarian activity, and that therefore the electric methods hitherto proposed are unsuitable for the detection of ovulation.

We are greatly indebted to Dr. Hallowell Davis for valuable suggestions during the course of these investigations.

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RÔLE OF THE KIDNEYS IN THE RESISTANCE OF RATS TO HEMORRHAGE

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Recent work has suggested that the kidney may play a rôle in resisting shock due to hemorrhage. This concept is supported by the following observations: 1, renal venous blood obtained from dogs which have been bled causes a greater increase in blood pressure than does similar blood from normal animals when injected into a recipient nephrectomized dog (1); 2, hemorrhage in nephrectomized dogs results in a lower blood pressure and a higher percentage of deaths than the same amount of blood loss in intact control animals (1); 3, in adrenalectomized dogs the renal mechanism seems essential for pressor activity (1); 4, blood from animals after hemorrhage, as tested on guinea-pig ileum, shows an increased content of angiotonin and a decreased content of renin-activator, as compared with a marked increase in renin-activator content in adrenalectomized-nephrectomized dogs (2); 5, blood pressure can be restored in dogs made hypotensive from hemorrhage by injection of renin-activator, presumably because secretion of renin after hemorrhage results in an exhaustion of renin-activator (3). The experiments here reported offer evidence that in the rat—as in the dog—the kidneys play a rôle in maintenance of blood pressure and prolongation of life in hypotension due to hemorrhage.

METHOD. In each experiment a pair of rats of the same sex and of approximately equal weight and age were used. These were given as nearly as possible identical treatment except that in the test animal the renal circulation was severed from its connection with the general circulation, while that of the control animal was left intact. A cannula was inserted into the abdominal aorta just proximal to its bifurcation and blood pressure readings were taken with a mercury manometer connected directly to the cannula. In five experiments the rats were anesthetized with 1 per cent sodium pentobarbital given intraperitoneally and later supplemented by intra-aortal injections of smaller quantities as necessary. In the other two experiments ether was used until approximately 0.5 cc. of 20 per cent paraldehyde in saline could be injected intra-aortally. Heparin in saline was administered to prevent clotting. Blood was withdrawn and injections made through a three-way tap connected to the aortic cannula.

Every effort was made to control the effects of such trauma as was necessary in order to carry out the experiments. Thus, in two experiments complete bilateral nephrectomy was performed through the abdominal cavity in the test animal, and renal decapsulation with as nearly as possiple the same amount of trauma was performed in the control animal. In one experiment the kidneys of both animals were freed from their capsule and peritoneal covering and ligatures

which had been passed around the pedicles were tied in the test animal and left loose in the control. In the remaining four experiments ligatures were passed around the vessels of both kidneys with a curved needle, avoiding trauma to the kidneys, and these were tied in the test animal and left loose in the control. The average duration of the operation was 20 minutes, so that there was relatively little difference in the duration of shock in the two animals, but in addition the order of operation on test and control rats was alternated in successive experiments. The technique of bleeding varied according to the amount required to give a moderate hypotension, but 0.5 cc. portions were removed every 10 minutes until roughly one per cent of total body weight was withdrawn and subsequently blood was withdrawn in 0.25 cc. portions until the animals died.

In one additional experiment adrenalectomy was performed through the abdomen in both test and control animals previous to the above-described operation and bleeding.

As a control to determine the effects of the anesthetic and operation alone two additional experiments were performed. A single rat under sodium pentobarbital anesthesia, without renal circulation, was allowed to remain without blood loss until death. In the second experiment a test and control animal, both under ether and paraldehyde, were allowed to remain similarly until death.

Results. The effects of blocking the renal circulation were striking in that in all experiments the control animal maintained a higher blood pressure after bleeding than did the test rat. This was true also in those experiments in which the initial blood pressure before bleeding was lower in the control than in the test animal. In all but one experiment the control rat lived longer (fig. 1 a–c), and in the single experiment in which the control animal died sooner, its blood pressure throughout the experiment, except for the last eleven minutes, was significantly higher than that of the test animal.

Results of the adrenalectomy were inconclusive since the first blood pressure readings indicated that the operation itself was extremely shocking, but the difference between test and control animals seen in the seven previous experiments were exaggerated in the adrenalectomized pair of rats. Both animals sustained hemorrhage poorly, that without renal circulation markedly less well (fig. 1, d). (In no experiment except that with adrenalectomy could any disturbance of the adrenals or their blood supply be demonstrated on gross postmortem examination.)

In the control experiments without hemorrhage the one animal under sodium pentobarbital without renal circulation maintained a blood pressure above 90 mm. Hg for $4\frac{1}{2}$ hours. In the two animals under paraldehyde the animal without renal circulation maintained a pressure above 95 for 3 hours, while the control had a consistently lower blood pressure but maintained it above 80 for $2\frac{2}{3}$ hours. This period was longer than the duration of hemorrhage and hypotension, from initial hemorrhage until death, in any of the experiments.

Discussion. Recent work has shown that the kidneys produce a pressor substance following hemorrhage which can be demonstrated by injection into dogs or by *in vitro* tests on guinea-pig ileum, and that nephrectomized dogs main-

tain a lower blood pressure than intact animals (1) (2). The experiments here reported show that rats deprived of their renal circulation withstand hemorrhage much more poorly than do control rats.

The following facts were considered to rule out other causes of the difference in response of test and control animals than the failure of the test animal to secrete a renal pressor substance: The effect of accumulated sodium pentobarbital, which is excreted mainly through the kidneys, was eliminated by experiments in

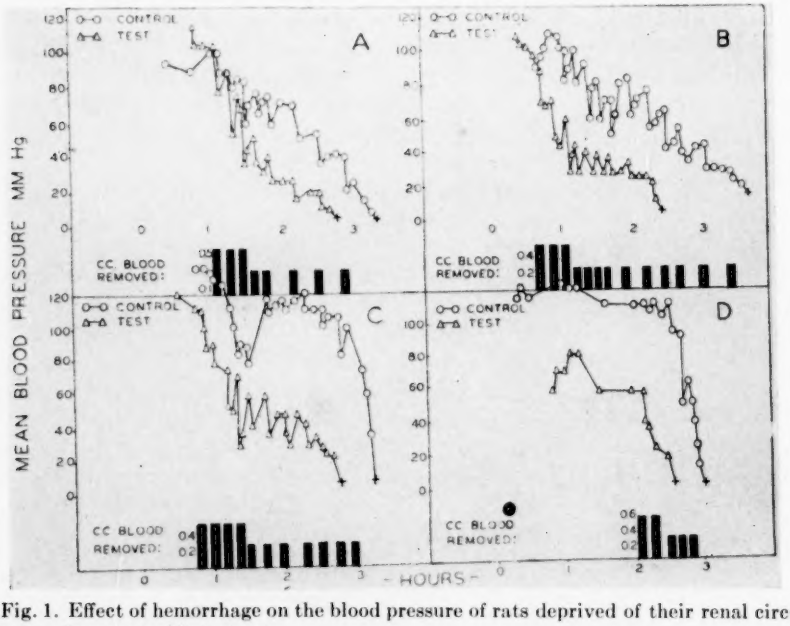

Fig. 1. Effect of hemorrhage on the blood pressure of rats deprived of their renal circulation and on control intact rats.

Fig. 1, a, b. Test animal had bilateral nephrectomy; control animal had renal decapsulation and sham nephrectomy. Both animals under nembutal.

Fig. 1, c. Ligatures passed around renal pedicle of both rats with a curved needle, tied in the test rat and left loose in the control. Both animals under nembutal.

Fig. 1, d. Both animals adrenalectomized. Ligatures passed around renal pedicles of both animals, tied in test animal and left loose in control. Both animals under ether followed by paraldehyde.

which ether and paraldehyde, which are eliminated mainly by the lungs (4), were employed as anesthetics. That the results were due to trauma or removal of the adrenals from the circulation was not considered tenable because of the separateness of the kidneys and adrenals in rats, the failure to demonstrate a damaged blood supply or damaged gland at post-mortem, the much greater shock caused by adrenalectomy than by nephrectomy, and by the fact that the action of the adrenal and kidney seemed to be additive in that adrenalectomized rats without renal circulation withstood hemorrhage extremely poorly as compared with the

control adrenalectomized rat. Although in these experiments no attempt was made to rule out reflexes arising from the kidney as a cause for the results, Hamilton and Collins have shown that the production of a pressor material does not depend entirely on connections with the central nervous system (1).

These experiments do not prove that the renin—renin-activator system, which has been shown to undergo changes during hemorrhage and shock, is responsible for the maintenance of blood pressure in this condition, but such seems the most logical explanation at the present time of the fact that animals deprived of their renal circulation withstand hemorrhage much less well than control intact animals.

SUMMARY

Rats anesthetized with sodium pentobarbital were subjected to repeated hemorrhage, the kidneys being removed from the circulation in one-half of the animals and being intact in the remainder. The nephrectomized animals displayed a greater drop in blood pressure, and died following the removal of a smaller amount of blood than the controls. The effects were not due to trauma to the adrenals because they were not modified when the kidneys of the control animals were manipulated to a degree equal to that required for a nephrectomy. Likewise, the results were not due to failure of excretion of the anesthetic agent because similar effects were observed in animals anesthetized with ether and paraldehyde. The observations support the concept, advanced by others, that the renal pressor mechanism may play a rôle in resistance to hemorrhage and other states attended by acute lowering of the blood pressure.

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THE MEASUREMENT OF BLEEDING VOLUME IN THE DOG FOR STUDIES ON BLOOD SUBSTITUTES[1]

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That the response to withdrawal of small volumes of blood changes with the state of the animal and with the volume and composition of the circulating fluid is a matter of common observation. There have been occasional attempts to use this as a means of gauging the severity of cardiovascular damage (Ebert, Stead, Warren and Watts, 1942) or the adequacy of blood substitutes (Gordon, Hoge and Lawson, 1942; Levinson, Weston, Janota and Necheles, 1943). The significance of such studies is obscure, since there has been no systematic investigation of the factors which limit the volume of blood which can be withdrawn from an animal at any particular circulatory level, and since the limiting factors cannot be predicted on *a priori* grounds.

It may be assumed with some assurance, however, that the volume obtained when an animal is bled to death depends upon the volume and the composition of the blood at the start of the bleeding; upon the amount of extravascular fluid which can be brought in, and the efficiency of mechanisms which maintain flow through vital organs, during the bleeding; and upon the ability of vital organs to survive reduction of blood flow toward the end of the bleeding. It appears likely that the relative importance of these factors will vary with the rate of bleeding. Rigorous control of bleeding rate is therefore essential if any interpretation of results is to be attempted. The term bleeding volume will be used in this report, unless it is qualified, to mean the volume of blood obtained by bleeding to death at controlled rates of blood withdrawal.

Comparison of bleeding volumes in groups of differently treated animals might be expected to yield useful information, but the value of the information gained would hardly justify either the labor or the large expenditure of animals which would be required. Data on the factors which govern bleeding volume could be obtained easily and economically if it were possible to repeat the measurement in the same animal at appreciable time intervals. Such repeated measurements are possible if a reliable end-point for the first exsanguination can be found, which will permit extrapolation to death with a fair amount of accuracy. It is obvious that even small blood withdrawals for the production of such an end-point may produce persistent changes in bleeding volume which will alter the value obtained in the second measurement. If irreparable damage is done to the cardiovascular system or its contents on the first withdrawal, reinjection of the drawn blood should yield low second values. If, on the contrary, there is a net gain of circulating fluid during the first withdrawal, and this fluid remains available, second volumes should be higher than the first.

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The studies reported here were undertaken in an attempt to find conditions under which bleeding volume could be determined twice in the same animal, the animal being killed by bleeding on the second test. Since the immediate object was the development of tests for the empirical comparison of blood substitutes (Lawson and Rehm, 1943), conditions were sought under which the second volume would be nearly the same as the first if the blood drawn for the first test was reinjected, and would be very low if no replacement was made. Similar considerations influenced the choice of a 4-hour interval between the first and the second measurement.

METHODS. Dogs kept without food and water for 18 to 24 hours were anesthetized by a single intravenous injection of sodium barbital, 250 mgm./kgm. A tracheal cannula was inserted, a carotid artery cannulated for recording mean arterial pressure, a femoral artery cannulated for drawing blood, and the ipselateral femoral vein prepared for infusions. Animals were not selected for size, sex, or condition, except that no obviously sick, immature, or pregnant animals were used. About one-fourth of the experiments were done with 0.9 per cent NaCl solution in the carotid cannula and blood-pressure line, with $\frac{1}{2}$ cc. 5 per cent chlorazol fast pink solution injected into the cannula as anticoagulant, the remainder with 5 per cent sodium citrate solution. No difference was observed in the results. The animals were kept in a supine position throughout the period of observation.

Blood was drawn by free arterial hemorrhage in unit volumes equal always to 2 cc./kgm. body weight at intervals of 1 to 4 minutes, the interval between withdrawals being kept constant throughout an experiment. The withdrawal of this volume usually required 5 to 10 seconds, except on the last few bleedings before death, which sometimes required 45 seconds or longer. The relatively long period in each interval during which no blood was flowing was utilized for flushing out the cannula to prevent clotting. The arterial pressure recorded as resulting from a unit volume withdrawal was read at the end of the interval, i.e., just before beginning the next withdrawal.

Heparinization of the drawn blood for reinjection was done by placing in the bleeding cannula before each unit withdrawal enough heparin solution to treat the volume to be drawn. The heparinized blood was filtered through cheesecloth just before infusion. Infusions were started immediately at the end of the interval in which the pre-arranged end-point was reached. No careful control of infusion rate was attempted, the blood being run in rapidly at first until arterial pressure began to rise, then more slowly, the whole infusion being completed within 3 to 8 minutes.

Of the several criteria considered for determining the exact time of death, the most satisfactory was found to be persistent depression of arterial pressure below 10 mm. Hg. To ensure uniformity in handling temporary depressions below this level, it was decided not to begin a bleeding if pressure at the time was below 10 mm., but not to discontinue a bleeding which had been started for any other reason than inability to obtain blood. Fractional withdrawals were thus obtained only on the last bleeding. They were counted as complete

if more than half the required volume was drawn, and not counted if less than half was obtained. The volumes reported are for this reason always multiples of 2 cc./kgm., and are accurate within 1 cc./kgm.

RESULTS. *Preliminary bleeding.* Exploratory studies made it apparent that the conditions sought for the comparison of blood substitutes could not readily be obtained by a simple double-hemorrhage procedure. If the first bleeding was terminated when death seemed imminent, and no replacement was made, arterial pressure not infrequently recovered, and fairly large second bleeding volumes were obtained 4 hours later. Replacement with saline after the first bleeding rather consistently yielded large second bleeding volumes. It was found, however, that neither spontaneous nor saline-assisted recovery of bleeding volume occurred following the second hemorrhage, if it was stopped short of death. Third bleeding volumes under these conditions were consistently and strikingly reduced, many of the animals dying within the 4 hours.

Since comparison of the first and the second volume did not appear to be useful for the present purposes, it was decided to use the first bleeding as a part of the preparation of the animals, and actually to compare the volumes obtained on the second and the third bleeding. To reduce the tedium of the procedure, the preparatory bleeding was done by withdrawing from all the animals a fixed volume of 20 cc./kgm. at the rate of 2 cc./kgm./min., from 2 to 5 hours before beginning the studies reported below. Since fixed volumes were drawn in the preliminary bleeding, with undetermined amounts of circulatory impairment, it cannot be considered a part of the bleeding volume determinations in these studies. All the studies reported here were done on animals who had survived this type of preparatory exsanguination, without replacement, 2 to 5 hours previously. It has not been possible to correlate any of the results with the length of time between preparatory bleeding and the first measurement, within these limits.

Selection of an end-point for the bleeding in the first measurement. The procedure as contemplated in theory requires that some easily recognized level of circulatory impairment be found which will lie a reasonably constant bleeding volume short of the death of the animal. The residual bleeding volume could be predicted at this point on the basis of control studies, without actually withdrawing the residual volume in animals who must be saved for second measurements.

In animals prepared as described above and bled to death at controlled rates between 2 cc./kgm./min. and 2 cc./kgm./4 min., records of mean arterial pressure had the characteristics shown in figure 1. Both the initial and the terminal plateau were observed with all rates, and were also obtained in animals who had not been subjected to preparatory bleeding. The plateaus are the result of progressive restoration of pressure during the intervals between unit withdrawals. Such restoration does not occur during the steeply-sloped middle portion of the record. In this portion of the bleeding, pressure usually continues to fall after a 2 cc./kgm. unit withdrawal is complete. These relationships are more apparent with the slower rates of bleeding.

Table 1 summarizes the data on animals bled to death at the 2 cc./kgm./3 min.

Fig. 1. Effect of hemorrhage at the rate of 2 cc./kgm./3 min. on arterial pressure. Preliminary hemorrhage 20 cc./kgm. at the rate of 2 cc./ kgm./min. 4 hours previously. Each numbered signal marks withdrawal of 2 cc./kgm. Time in minutes. Progressive diminution in the amplitude of Mayer waves on the initial plateau is typical.

TABLE 1

Distribution of the volume of blood drawn during fatal hemorrhage with reference to the terminal plateau in arterial pressure (see text and fig. 1)

Hemorrhage rate 2 cc./kgm./3 min. The volume drawn on preliminary hemorrhage, 20 cc./kgm., is not included

DOG NO.	BEGINNING OF TERM. PLAT.	VOL. DRAWN TO BEGINNING OF TERM. PLATEAU	VOL. DRAWN ON TERM. PLATEAU	TOTAL VOL. DRAWN
	mm. Hg	*cc./kgm.*	*cc./kgm.*	*cc./kgm.*
1	26	18	14	32
2	40	20	16	36
3	36	18	16	34
4	42	26	14	40
5	41	22	20	42
6	33	28	16	44
7	47	12	20	32
8	34	20	12	32
9	44	20	20	40
10	47	22	20	42
11	40	16	12	28
12	44	4	16	20
13	36	2	12	14
14	38	6	14	20
15	36	8	16	24
16	45	6	14	20
17	55	8	14	22
Mean			15.65	30.71
S.D.			*2.85*	*9.35*
S.E.			*0.69*	*2.27*

if more than half the required volume was drawn, and not counted if less than half was obtained. The volumes reported are for this reason always multiples of 2 cc./kgm., and are accurate within 1 cc./kgm.

RESULTS. *Preliminary bleeding.* Exploratory studies made it apparent that the conditions sought for the comparison of blood substitutes could not readily be obtained by a simple double-hemorrhage procedure. If the first bleeding was terminated when death seemed imminent, and no replacement was made, arterial pressure not infrequently recovered, and fairly large second bleeding volumes were obtained 4 hours later. Replacement with saline after the first bleeding rather consistently yielded large second bleeding volumes. It was found, however, that neither spontaneous nor saline-assisted recovery of bleeding volume occurred following the second hemorrhage, if it was stopped short of death. Third bleeding volumes under these conditions were consistently and strikingly reduced, many of the animals dying within the 4 hours.

Since comparison of the first and the second volume did not appear to be useful for the present purposes, it was decided to use the first bleeding as a part of the preparation of the animals, and actually to compare the volumes obtained on the second and the third bleeding. To reduce the tedium of the procedure, the preparatory bleeding was done by withdrawing from all the animals a fixed volume of 20 cc./kgm. at the rate of 2 cc./kgm./min., from 2 to 5 hours before beginning the studies reported below. Since fixed volumes were drawn in the preliminary bleeding, with undetermined amounts of circulatory impairment, it cannot be considered a part of the bleeding volume determinations in these studies. All the studies reported here were done on animals who had survived this type of preparatory exsanguination, without replacement, 2 to 5 hours previously. It has not been possible to correlate any of the results with the length of time between preparatory bleeding and the first measurement, within these limits.

Selection of an end-point for the bleeding in the first measurement. The procedure as contemplated in theory requires that some easily recognized level of circulatory impairment be found which will lie a reasonably constant bleeding volume short of the death of the animal. The residual bleeding volume could be predicted at this point on the basis of control studies, without actually withdrawing the residual volume in animals who must be saved for second measurements.

In animals prepared as described above and bled to death at controlled rates between 2 cc./kgm./min. and 2 cc./kgm./4 min., records of mean arterial pressure had the characteristics shown in figure 1. Both the initial and the terminal plateau were observed with all rates, and were also obtained in animals who had not been subjected to preparatory bleeding. The plateaus are the result of progressive restoration of pressure during the intervals between unit withdrawals. Such restoration does not occur during the steeply-sloped middle portion of the record. In this portion of the bleeding, pressure usually continues to fall after a 2 cc./kgm. unit withdrawal is complete. These relationships are more apparent with the slower rates of bleeding.

Table 1 summarizes the data on animals bled to death at the 2 cc./kgm./3 min.

Fig. 1. Effect of hemorrhage at the rate of 2 cc./kgm./3 min. on arterial pressure. Preliminary hemorrhage 20 cc./kgm. at the rate of 2 cc./ kgm./min. 4 hours previously. Each numbered signal marks withdrawal of 2 cc./kgm. Time in minutes. Progressive diminution in the amplitude of Mayer waves on the initial plateau is typical.

TABLE 1

Distribution of the volume of blood drawn during fatal hemorrhage with reference to the terminal plateau in arterial pressure (see text and fig. 1)

Hemorrhage rate 2 cc./kgm./3 min. The volume drawn on preliminary hemorrhage, 20 cc./kgm., is not included

DOG NO.	BEGINNING OF TERM. PLAT.	VOL. DRAWN TO BEGINNING OF TERM. PLATEAU	VOL. DRAWN ON TERM. PLATEAU	TOTAL VOL. DRAWN
	mm. Hg	*cc./kgm.*	*cc./kgm.*	*cc./kgm.*
1	26	18	14	32
2	40	20	16	36
3	36	18	16	34
4	42	26	14	40
5	41	22	20	42
6	33	28	16	44
7	47	12	20	32
8	34	20	12	32
9	44	20	20	40
10	47	22	20	42
11	40	16	12	28
12	44	4	16	20
13	36	2	12	14
14	38	6	14	20
15	36	8	16	24
16	45	6	14	20
17	55	8	14	22
Mean			15.65	30.71
S.D.			*2.85*	*9.35*
S.E.			*0.69*	*2.27*

rate. If the 20 cc./kgm. drawn from each animal on the preparatory bleeding were added to the volumes recorded in the table, the total volumes obtained and the individual variations would be of the same order of magnitude as those reported for unanesthetized animals bled to death by uncontrolled arterial hemorrhage (Ivy, Greengard, Stein, Grodins and Dutton, 1943). The volume drawn on the terminal plateau is considerably more constant than the volume required to lower pressure to the plateau. The standard deviation of the former value, as shown in the table, is only 18.5 per cent of its mean, while the standard deviation of the total bleeding volume is 30.5 per cent of its mean. It appears from these data that in animals prepared and bled in this way, arrival at the terminal plateau signals production of a circulatory state which can withstand only limited and fairly constant further volume withdrawal. The constancy of length of the terminal plateau cannot be ascribed simply to the fact that all the animals are exposed for the same length of time to damage at these low pressure levels, since animals bled to the expected middle of the plateau and left undisturbed not infrequently lived for 2 hours or longer without either a rise or a fall in pressure. Residual bleeding volume could be predicted with reasonable accuracy on the basis of the data of table 1 if the actual bleeding during the first measurement were terminated anywhere along the lower plateau, at fixed volumes from its beginning.

Although the terminal plateau is easy to identify on most finished records, it is often difficult to demonstrate on tracings which are in progress without invading it for a number of unit bleedings. If the plateau is to be used as an empirical landmark for terminating the first bleeding, it seemed better not to invade it, but to try to obtain a pressure end-point lying just above its beginning on the steeply-sloped portion of the record. Animals were accordingly bled to death at various rates, and an examination made of the constancy of volume withdrawal required to produce death after pressure had been lowered to 60, 50, and 40 mm. Hg. The data for three rates of bleeding are summarized in table 2. Bleeding volume is divided in the table into: H_{1a}, the volume required to lower pressure to the level chosen as trial end-point; and H_{1c}, the volume required to produce death after this level had been passed. Since pressure may continue to fall after a 2 cc./kgm. unit withdrawal is complete, the volumes given in the table are the volumes required to lower pressure below the trial pressure level. The table does not include animals whose pressure was reduced below 60 mm. by withdrawal of less than 12 cc./kgm. They do not appear to be suitable for studies on blood substitutes for reasons which are considered in a subsequent section of this report, and are excluded from all summaries of data except tables 1 and 5. Comparison of the data on the 3-minute rate in the case of those animals which appear in both table 1 and table 2 shows that the 60 mm. end-point usually lies just above the beginning of the plateau, while the 50 mm. end-point is in some cases above, in other cases below this level.

The accuracy with which the first bleeding volume could be obtained by withdrawing blood to any of these pressure end-points and extrapolating for the residual volume, appears from the data of table 2 to decrease as the rate of

bleeding increases. The percentage error would be greatest when minimum values for H_{1a} (actual volume drawn) happened to coincide with maximum deviations of H_{1e} (residual volume) from its mean. If 60 mm. Hg is used as the level for terminating the actual withdrawal (it appears from table 2 to be the most reliable pressure end-point for most of the rates); if minimum H_{1a} volumes are 12 cc./kgm., and if 2 s.d. be taken as the greatest deviation from the mean

TABLE 2

The volume of H_{1a} and H_{1e} obtained at various levels of arterial pressure

All volumes as cc./kgm. Only mean values with standard deviation and standard error are given, except for first group. See text for explanation of symbols.

DOG NO.	RATE HEM.	VOLUME H_{1a} WHEN END-POINT IS:			VOLUME H_{1e}, MEASURED FROM END-POINT		
		60 mm. Hg	50 mm. Hg	40 mm. Hg	60 mm. Hg	50 mm. Hg	40 mm. Hg
1	2 cc./kgm./3 min.	14	16	20	18	16	12
2	2 cc./kgm./3 min.	18	22	26	18	14	10
3	2 cc./kgm./3 min.	14	16	20	20	18	14
4	2 cc./kgm./3 min.	26	28	30	14	12	10
5	2 cc./kgm./3 min.	22	22	24	20	20	18
6	2 cc./kgm./3 min.	22	26	28	22	18	16
7	2 cc./kgm./3 min.	14	14	20	18	18	12
8	2 cc./kgm./3 min.	20	20	20	12	12	12
9	2 cc./kgm./3 min.	18	20	24	22	20	16
10	2 cc./kgm./3 min.	20	24	36	22	18	6
11	2 cc./kgm./3 min.	14	16	18	14	12	10
Mean		18.36	20.36	24.18	18.18	16.18	12.36
S.D.		*4.08*	*4.55*	*5.47*	*3.51*	*3.16*	*3.45*
S.E.		*1.20*	*1.37*	*1.65*	*1.06*	*0.95*	*1.04*
NO. DOGS							
7	2 cc./kgm./2 min.	21.71	23.14	25.14	16.57	15.14	13.14
S.D.		*8.20*	*7.90*	*8.23*	*4.56*	*4.73*	*5.02*
S.E.		*3.09*	*2.98*	*3.10*	*1.72*	*1.78*	*1.89*
7	2 cc./kgm./4 min.	18.57	20.86	22.0	10.57	8.29	7.14
S.D.		*3.41*	*3.80*	*4.61*	*1.90*	*3.15*	*2.54*
S.E.		*1.29*	*1.44*	*1.74*	*0.72*	*1.19*	*0.96*

value of H_{1e}, the error will not in any case exceed 17 per cent for the 4-minute rate, 23 per cent for the 3-minute rate, and 32 per cent for the 2-minute rate.

The unavoidable change in bleeding volume following the first measurement. Table 3 summarizes the results obtained when the blood drawn for H_{1a} measurement was reinjected as soon as the end-point was reached. The estimated residual volumes at the end-point for the first bleeding are the H_{1e} values of table 2. The second bleeding volumes (H_2) were measured about 4 hours after completing the replacement of blood, and were obtained by bleeding the animals

completely to death, always at the rate used for the first measurement. The H_2:H_1 ratios obtained, where H_1 is taken as the sum of H_{1a} and H_{1e}, have been multiplied by 100 for the table to show the percentage change in bleeding volume under these conditions.

The variations in H_2:H_1 appear from the table to become increasingly large as slower rates of bleeding are used for the measurements. Since the accuracy in estimating H_{1e} increases as slower rates are used (see table 2), these variations can hardly be attributable entirely to the error in computing H_1. For the 2-minute rate, the variations are almost exactly those to be expected from the

TABLE 3

$\frac{H_2}{H_1} \times 100$ *when the blood drawn for measurement of* H_{1a} *is heparinized and reinjected as soon as the measurement is complete*

H_1-H_2 interval approximately 4 hours. All volumes in cc./kgm. H_{1e} values obtained from table 2. See text for explanation of symbols.

NO. ANIMALS	RATE HEM.	END-PT. H_{1a}	VOLUME H_{1a}	VOLUME H_{1e}	VOLUME H_2	$\frac{H_2}{H_1} \times 100$
		mm. Hg				
10	2 cc./kgm./2 min.	40	22.4	13.14	31.0	87.4
S.D.			*6.10*		*6.27*	*7.16*
S.E.			*1.96*		*1.98*	*2.26*
8	2 cc./kgm./3 min.	60	17.75	18.18	26.25	73.6
S.D.			*3.77*		*5.26*	*13.82*
S.E.			*1.43*		*1.99*	*5.23*
10	2 cc./kgm./3 min.	60*	23.0	14.18	26.2	69.9
S.D.			*4.32*		*6.35*	*11.54*
S.E.			*1.37*		*2.01*	*3.65*
5	2 cc./kgm./4 min.	60	17.2	10.57	20.0	70.6
S.D.			*5.40*		*9.16*	*30.08*
S.E.			*2.41*		*4.09*	*13.43*

* + 4 cc./kgm.

error in H_1, but for both the slower rates the scatter in H_2:H_1 is much too great to be accounted for in this way. Since the values for H_2:H_1 decrease as well as scatter when the slower rates are used, it is tempting to suppose that the slower rates introduce additional variables in the form of some sort of irreversible cardiovascular damage. Neither the mean ratio nor the magnitude of its variations was significantly altered, however, when bleeding at the 3-minute rate was continued until an extra 4 cc./kgm. had been drawn, before replacement (table 3).

The extent of spontaneous recovery when the blood drawn for the first measurement

is not replaced. If changes in bleeding volume are to be used empirically for the evaluation of blood substitutes, it is obviously important to show that low second volumes are obtained when no replacement is made after withdrawal for the first measurement. The experiments of table 3 were accordingly repeated without any replacement after the H_{1a} volumes had been withdrawn. Studies of this sort were not done for the 4-minute rate, since the variations in $H_2:H_1$ obtained with it in the foregoing appeared to be too large for the present purposes. The data are given in table 4. Of the 30 animals listed in the table, only 11 survived the 4-hour interval. Only 3 of these had bleeding volumes at the end of 4 hours which were larger than the residual volumes estimated

TABLE 4

$\frac{H_2}{H_1} \times 100$ *when no replacement is made after withdrawing* H_{1a}

H_1–H_2 interval approximately 4 hours. All volumes in cc./kgm. H_{1e} values obtained from table 2. Symbols as in table 3.

NO. ANIMALS	RATE HEM.	END-PT. H_{1a}	VOLUME H_{1a}	VOLUME H_{1e}	VOLUME H_2	$\frac{H_2}{H_1} \times 100$
		mm. Hg				
10	2 cc./kgm./2 min.	40	20.2	13.14	4.8	15.2
	S.D.		*3.19*		*6.74*	*21.38*
	S.E.		*1.01*		*2.13*	*6.75*
10	2 cc./kgm./3 min.	60	22.8	18.18	7.2	19.6
	S.D.		*8.44*		*8.22*	*21.36*
	S.E.		*2.67*		*2.60*	*6.72*
10	2 cc./kgm./3 min.	60*	25.6	14.18	2.6	6.2
	S.D.		*5.32*		*5.48*	*13.12*
	S.E.		*1.37*		*1.73*	*4.15*

* + 4 cc./kgm.

for them on completing the first bleeding. In none of these did the second volume exceed the value taken for H_{1e} by more than 0.5 s.d.

It is apparent from these data that if a spontaneous rise in bleeding volume above its residual value occurs after withdrawal of H_{1a}, it does not persist for 4 hours. It would appear, therefore, that any value for H_2 in excess of the value for H_{1e}, may under these experimental conditions be attributed to the type of treatment given after withdrawal of H_{1a}.

The behavior of animals with small control bleeding volumes. Six animals appear in table 1 whose arterial pressure was reduced to the terminal plateau on withdrawal of less than 12 cc./kgm. Plateau volumes are somewhat less for them (average 14.33 cc./kgm.) than for the rest of the animals (16.36 cc./kgm.), but are within the limits of variation for the latter. It has become apparent in the course of these studies that animals with very small bleeding volumes

above the plateau constitute a special group. $H_2:H_1$ ratios obtained for them in experiments with reinjected blood and with other fluids are often beyond the limits of variation for the rest of the animals (Lawson and Rehm, loc. cit.). Illustrative data are given in table 5, for replacement with blood. The limits of variation for the other animals in the respective groups are estimated in the table as the mean values found in table 3 plus and minus 2 s.d.

It may be supposed that occasional animals in this group have had arterial pressure lowered to the end-point selected for H_1 measurement by other mechanisms than simple blood withdrawal. Their residual bleeding volumes at the end of H_{1a} withdrawal would probably be excessively large, and they might be expected to yield excessively high $H_2:H_1$ ratios. It may be further supposed that the rest of them are in a state of unusually rapid deterioration. For the present purposes, both types appear to be excluded by confining the studies to animals who require at least 12 cc./kgm. blood withdrawal to lower pressure to 60 mm. on the first bleeding volume determination. After the animals with

TABLE 5

Individual $H_2:H_1$ ratios obtained in autotransfusion experiments on 4 animals whose arterial pressure was lowered to 60 mm. Hg by withdrawal of less than 12 cc./kgm. on H_1 measurement

HEM. RATE	H_{1a} END-POINT	VOL. LOSS AT 60 MM. Hg ON H_1	$\frac{H_2}{H_1} \times 100$	MEAN RATIO FOR GROUP ±2 S.D.
	mm. Hg	*cc./kgm.*		
2 cc./2 min.	40	4	0	101.7–73.1
2 cc./3 min.	60	6	34	101.2–46.0
2 cc./3 min.	60*	6	49	93.0–46.8
2 cc./4 min.	60	6	162	130.7–10.5

* + 4 cc./kgm.

H_{1a} volumes smaller than this have been excluded, the $H_2:H_1$ ratios obtained do not appear to be related to the control bleeding volumes (Lawson and Rehm, loc. cit.). In these studies about one-sixth of the animals have been discarded because of small H_{1a} volumes. This includes animals who died before the first bleeding volume measurement could be made. A total of 4 animals in addition (about 1 per cent) have been excluded because the first bleeding record suggested that the terminal plateau had been invaded by 6 cc./kgm. or more in lowering pressure to 60 mm. In 3 of these animals aberrant low $H_2:H_1$ ratios were obtained. Lesser invasion of the terminal plateau appears from the records to have occurred in a number of instances without significantly affecting the value of $H_2:H_1$.

Discussion. These data show that control measurements of bleeding volume may be made under certain conditions without producing unpredictable persistent changes in the value of a subsequent measurement. The factors which govern resistance to fatal hemorrhage thus appear to be susceptible to laboratory investigation, since the change in resistance under a given set of conditions may be studied in a single animal.

The primary purpose in these studies was not the measurement of absolute values for bleeding volume in normal animals. Such a purpose would have been defeated by the use of anesthesia and by the use of a preparatory fixed-volume bleeding with undetermined amounts of cardiovascular damage. The studies were designed to find conditions under which the change in bleeding volume between the first and the second measurement is a function of the circulating fluid replacement made at the end of the first. The data given here do not prove that these conditions have been found, since they test only the two extremes of replacement, no infusion and infusion of all the blood drawn for the first measurement. Data are given elsewhere (Lawson and Rehm, loc. cit.) which show that the amount of change in bleeding volume between first and second measurement depends upon the type of fluid given in replacement.

Economy of expression in subsequent discussions of bleeding volume may be served by use of the following symbols:

H_1 = Control bleeding volume. It is measured at a stated, controlled rate of bleeding, in animals prepared under standard conditions. It is obtained by drawing blood to a stated level of circulatory impairment (in these studies a level of arterial pressure near the beginning of the terminal plateau) and is equal to the sum of the actual volume drawn in reaching this end-point (H_{1a}) plus the residual volume (H_{1e}). The residual volume is obtained from control animals by continuing the first bleeding past the stated end-point to death. In the studies reported here for the value of H_{1e}, an attempt has been made to obtain a fair sample of the animals to be used, with respect to size, sex, and H_{1a} values. Seasonal and nutritional influences on the value of H_{1e} have not been studied.

R = Fluid given after H_{1a} has been drawn.

H_2 = Second bleeding volume. Unless otherwise stated, it is always measured by drawing blood at the rate used for H_1 measurement, and is continued without interruption to the death of the animal. If it is to be estimated from a partial bleeding volume, as in the case of H_1, studies will have to be made on the reliability of end-point.

H_1–H_2 Interval = The interval between arrival at the end-point for H_1 measurement (in these studies R was infused at this time), and the beginning of H_2 measurement.

It is not surprising that bleeding volume declines during the period of observation in these studies with all the rates of bleeding used, so that H_2:H_1 is almost always less than 1, even when R is the blood drawn for H_{1a} measurement. The animals had been kept without food and water for 18 to 24 hours, were under barbital anesthesia, and had been subjected to a preparatory hemorrhage of 20 cc./kgm. before beginning the studies. Damage to the cardiovascular system which would result in irretrievable fluid loss or to impairment of vital organs might be expected to occur under these conditions, and to result in progressive decline in bleeding volume even though the partial exsanguination required for H_1 measurement did not in itself do any irreversible damage. That the measured decline is in some way related to the rate of bleeding used in the determinations seems clear from the data. Whether this means that more irreversible damage is done by the slower hemorrhages when they are used for drawing H_{1a}, as it might appear to mean, cannot be decided without further study. It may mean only that different mechanisms control the volumes obtained with slow and

with fast bleeding, and that the rate of deterioration of these mechanisms is not identical.

The 2 cc./kgm./3 min. rate of bleeding has been selected on the basis of these data for use in the studies on blood substitutes. It is a convenient rate to use. The mechanisms which govern the volume obtained with it appear to undergo fairly rapid deterioration under these experimental conditions, since H_2 is only about 70 per cent of H_1 after an H_1–H_2 interval of 4 hours, when R is the blood drawn for H_{1a} measurement. The rate of deterioration appears to be relatively constant for all animals. Low values for H_2, with average H_2:H_1 ratios approaching 0, are obtained if R is 0.

SUMMARY

Procedures are described which permit bleeding volume, i.e., the volume withdrawal at controlled rates required to produce death, to be measured with usable accuracy without actually killing the animal. The residual volume, which is not drawn on the first measurement, is estimated with increasing accuracy as the rate of bleeding is reduced from 2 cc./kgm./2 min. to 2 cc./kgm./4 min. Second bleeding volumes, measured 4 hours after replacing the blood drawn on the first test, and obtained by withdrawing blood at the same rate until the animal dies, were almost always somewhat lower than the controls under the experimental conditions described. Second bleeding volumes were in the neighborhood of 90 per cent of the controls when the 2 cc./kgm./2 min. rate was used, and in the neighborhood of 70 per cent when the 2 cc./kgm./3 min. rate was used. When the blood drawn for the control measurements was not replaced, second volumes 4 hours later were never significantly larger than the residual volumes estimated at the termination of the control tests, and were usually considerably smaller, with average values approaching 0.

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THE RELATIVE VALUE OF VARIOUS FLUIDS IN REPLACEMENT OF BLOOD LOST BY HEMORRHAGE, WITH SPECIAL REFERENCE TO THE VALUE OF GELATIN SOLUTIONS[1]

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Procedures have been described which permit bleeding volume, i.e., the volume of blood withdrawal required at controlled rates to produce death, to be measured twice in the same animal; and conditions have been found which yield relatively low second volumes when the blood drawn for the first test is not replaced (Lawson, 1943). An empirical comparison of blood substitutes may be made by substituting the fluid under investigation for the blood drawn in the first measurement, and comparing the $H_2:H_1$ ratios obtained.

The procedures selected for these studies yield second bleeding volumes somewhat lower than the controls at the end of 4 hours, if the blood drawn during the first test is heparinized and reinjected. The obvious conclusion has been drawn that some mechanism or mechanisms which govern resistance to fatal hemorrhage undergo deterioration under the conditions of the experiments. The $H_2:H_1$ ratios obtained when R (fluid replacement at end of blood withdrawal for first test) is some fluid other than blood cannot be used with assurance to show the influence of the substitute fluid on these same mechanisms. Bleeding volume is an empirical value which is probably influenced by a large number of physiological variables. Thus, if a foreign fluid yields higher $\frac{H_2}{H_1}$ ratios than are obtained when R is the blood drawn, it cannot be concluded that the fluid is actually superior to blood in its direct effect on all the mechanisms which govern bleeding volume. The rate of deterioration of some of these may actually be greater when the substitute is used. Similar difficulties of interpretation are encountered if other empirical values, such as mortality rates, are measured after limited intervals of time. These difficulties are not obviated by measuring conventional values, such as blood volume, cardiac output, or circulatory pressures, since none of them can be regarded as testing an isolated mechanism.

The information obtained by comparing the $\frac{H_2}{H_1}$ ratios appears to provide as legitimate a comparison of blood substitutes as could be obtained by subjecting animals to hemorrhage which would be expected to cause death, injecting the fluid under investigation, and determining the percentage of the group which is still alive at the end of a few hours (Ivy, Greengard, Stein, Grodins and Dutton, 1943; Wiggers, 1943). Since a measured value is obtained for each animal, statistically reliable data are obtained with fewer animals than in

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survival rate studies which determine only whether an animal is alive or dead at the end of a fixed time interval. Furthermore, if the fluids are to be given to subjects who lose circulating fluid after the injection those fluids which yield the larger bleeding volumes in these tests might reasonably be expected to support life longer than those which yield small bleeding volumes. This would not necessarily be true if, under conditions of clinical use, types of circulatory damage are encountered which are aggravated by the fluids, and which are not produced in the animals used for the tests. In these studies any simulation of clinical states in which blood substitutes might be employed is incidental.

METHODS. The procedure already described in detail (Lawson, loc. cit.) was carried out on barbitalized dogs without modification except that the fluid under investigation was substituted for autotransfused blood in the replacement (R) following the first bleeding volume measurement. The volume of R was always equal to the volume of blood actually drawn during the first measurement (H_{1a}). Blood withdrawal for both H_1 and H_2 determination was done at the rate of 2 cc./kgm./3 min. On H_1 determination actual blood withdrawal was discontinued as soon as arterial pressure fell below 60 mm. Hg. The residual bleeding volume at this time (H_{1e}) was taken from the previously reported control studies as 18.18 cc./kgm., and H_1 was computed as the sum of H_{1a} and H_{1e}. The H_1–H_2 interval was approximately 4 hours. Measurement of H_2 was made by bleeding the animal actually to death.

The blood, plasma and serum used in these studies were obtained by bleeding donor dogs to death under barbital anesthesia, pooling the freshly drawn blood from 5 to 8 donors before further processing. These fluids were chilled as soon as possible (serum and defibrinated blood as soon as clotting was complete) and refrigerated until used. They were used within 8 hours after obtaining the blood from the donors.

The two gelatins selected for study are oppositely charged colloids at the pH of blood.[2] Gelatin VII-54 is prepared from pork skin, and has an isoelectric point in the neighborhood of pH 8. A physically similar pork skin gelatin was studied by Ivy et al. (loc. cit.) under the designation U-17578. Gelatin B-78-1 is prepared from beef bone, and has an isoelectric point near pH 5. Its use as a blood substitute has been studied by Parkins and Lockwood, 1943; Little and Wells, 1943, and Little and Dameron, 1943. A similar gelatin was used in the preliminary studies of Gordon, Hoge and Lawson, 1942. The gelatins were dissolved in 0.9 per cent NaCl to a concentration of 3.45 per cent for B-78-1 and a concentration of 4.85 per cent for VII-54, and pH was adjusted to 7.0–7.3 by addition of N/10 NaOH. At these concentrations the solutions had a colloidal osmotic pressure of 22.5 mm. Hg, which was approximately the same as that of the hematogenous fluids used in the study. Colloidal osmotic pressures were measured at 37°C. by a modification of the method described by Hepp (1936), using tight membranes with an average permeability equal to 11.2×10^{-8} in the units recommended by Wells (1932).

RESULTS. The data obtained are summarized in table 1, which also includes,

[2] All supplies of gelatin were furnished by the Knox Gelatine Company, Johnstown, N. Y.

TABLE 1

$\frac{H_2}{H_1} \times 100$ *with different types of replacement fluid*

H_1–H_2 interval 4 hours. Values given are the means, with their standard deviations (S.D.) and standard errors (S.E.). See text for explanation of symbols in headings.

REPLACEMENT FLUID (R) VOL. = VOL. H_{1a}	NUMBER OF ANIMALS	H_{1a}	H_{1e}	H_2	$\frac{H_2}{H_1} \times 100$
		cc./kgm.	*cc./kgm.*	*cc./kgm.*	
0	10	22.8	18.18	7.2	19.6
S.D.		*8.44*		*8.22*	*21.36*
S.E.		*2.67*		*2.60*	*6.72*
Autotransfused blood	8	17.75	18.18	26.25	73.6
S.D.		*3.77*		*5.26*	*13.82*
S.E.		*1.43*		*1.99*	*5.23*
0.9% NaCl	10	21.6	18.18	14.8	37.6
S.D.		*4.81*		*7.73*	*18.07*
S.E.		*1.52*		*2.44*	*5.69*
Heparinized blood	10	20.6	18.18	27.2	70.7
S.D.		*4.21*		*5.09*	*13.57*
S.E.		*1.33*		*1.58*	*4.30*
Defibrinated blood	8	21.0	18.18	24.8	62.8
S.D.		*5.23*		*7.24*	*14.66*
S.E.		*1.85*		*2.56*	*5.18*
Heparinized plasma	10	19.4	18.18	25.8	68.1
S.D.		*4.52*		*5.99*	*9.87*
S.E.		*1.43*		*1.90*	*3.12*
Serum	9	16.4	18.18	24.4	70.5
S.D.		*7.06*		*6.98*	*16.28*
S.E.		*2.49*		*2.47*	*5.75*
Gelatin VII-54, 4.85%	8	23.0	18.18	25.3	60.5
S.D.		*8.48*		*9.55*	*16.03*
S.E.		*3.00*		*3.38*	*5.67*
Gelatin B-78-1, 3.45%	7	16.3	18.18	21.1	61.1
S.D.		*3.14*		*3.80*	*7.84*
S.E.		*1.18*		*1.43*	*2.96*

for comparison, previously reported data obtained when R is the blood drawn for H_{1a} measurement, and when R is 0 (no infusion).

The probability that random sampling variations account for the differences in $\frac{H_2}{H_1}$ obtained when R is 0.9 per cent NaCl and when R is 0 is approximately 0.06. The probability that the observed differences between 0.9 per cent NaCl and the remaining fluids are due to variations of this sort is in every case less than 0.02 (Fisher, 1941). Attempts were made to obtain fair samples by avoiding any selection of animals within the limits previously described, the animals being used in whatever studies were in progress as they were delivered to the laboratory. The possibility of seasonal variations in the value of H_{1e} has been suggested by more recent observations, and has not been fully explored. All the comparative data reported here were obtained within a single season, and determination of H_{1e} was done concurrently.

No statistically significant differences are observed in the table among the various colloidal solutions employed. The suggestively low values obtained with defibrinated blood require further study in view of the possibility that partially hemolyzed blood may be inferior as an infusion fluid (DeGowin, 1942).

Temporary depressor effects were usually obtained with pooled blood, plasma, and serum, but not with autotransfused blood. Neither the data of the table, nor examination of individual responses reveals any correlation between these effects and the $\frac{H_2}{H_1}$ ratios obtained.

Although the two gelatin solutions yield about the same mean value, much lower values for $\frac{H_2}{H_1}$ were obtained in some of the animals receiving VII-54 than were ever observed with B-78-1 (see s.d. in table). Whether this means that VII-54 has positive harmful effects in occasional animals, or simply that it fails to be retained as circulating colloid, cannot be decided without further study. The differences shown in the table between the gelatin solutions on the one hand and heparinized blood, plasma, and serum, on the other, are in general agreement with comparative data which have been obtained on one of them (VII-54) in the treatment of animals subjected to single massive hemorrhage, using 24-hour survival rates as an index to adequacy of blood substitution (Ivy et al., loc. cit.).

The relationship between $\frac{H_2}{H_1}$ *and the volume of* H_{1a}. It has already been shown that the magnitude of the ratio (i.e., the rate of decline in bleeding volume during the 4-hour H_1–H_2 interval) is not related to the control bleeding volume, if the latter is such that H_{1a} is 12 cc./kgm. or greater, in experiments in which R is the animal's own blood (Lawson, loc. cit.). It cannot be assumed, however, that this will also be true when R is a foreign fluid. The volume of fluid which each animal received is always, in the studies reported here, equal to the volume of H_{1a}. If the fluids given are toxic in the customary sense, the animals with the larger H_{1a} volumes and who receive correspondingly larger volumes of the fluids might be expected to yield the lower $\frac{H_2}{H_1}$ ratios. These relationships are examined

in figure 1 for the two gelatin solutions and for defibrinated blood, which might be regarded as most likely, of all the fluids studied, to possess some sort of harmful effect. The figure fails to show any relationship between the volume of H_{1a}, and therefore the volume of R, and $\frac{H_2}{H_1}$. Similar data have been obtained for the other fluids studied.

These data are not presented to show that harmful effects are absent, but simply that within the very limited dosage range covered by variations in the volume of R when the latter is made equal always to H_{1a}, the effects do not appear to influence the value of $\frac{H_2}{H_1}$. Comparisons such as those given in table 1

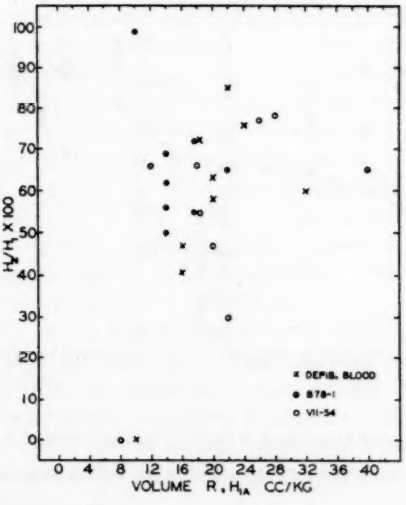

Fig. 1. Relationship between $\frac{H_2}{H_1}$ and the volume of R when R is equal to H_{1a}, in experiments on defibrinated blood (pooled), gelatin VII-54, 4.85 per cent in 0.9 per cent NaCl, and gelatin B-78-1, 3.45 per cent in 0.9 per cent NaCl.

are therefore valid, for all the fluids studied, even though identity of H_{1a} is not obtained with these small groups of animals.

The 3 animals shown in figure 1 whose H_{1a} volumes were less than 12 cc./kgm. demonstrate the wide variations in $\frac{H_2}{H_1}$ obtained when such animals are used, which were discussed in the previous report. Animals of this sort are not included in any other summaries of data in this report.

The value of various grades of gelatin. A series of increasingly degraded gelatins was prepared from a single high average mol. wt. gelatin, by exposing stock solutions of the latter to temperatures of 115.5°C. (10 lbs. autoclave pressure) for increasing lengths of time up to 3 hours. The stock solutions

were made from gelatin B-78-1, or from a duplicate manufacturer's batch. pH was adjusted to 7.4 before autoclaving, the pH range of the solutions after autoclaving being 6.9 to 7.3.

The data on this series are summarized in figure 2, in which the gelatin concentration of the prepared solutions was adjusted to 3.45 grams/100 cc. before use. It is apparent from the figure that complex changes occur in the value

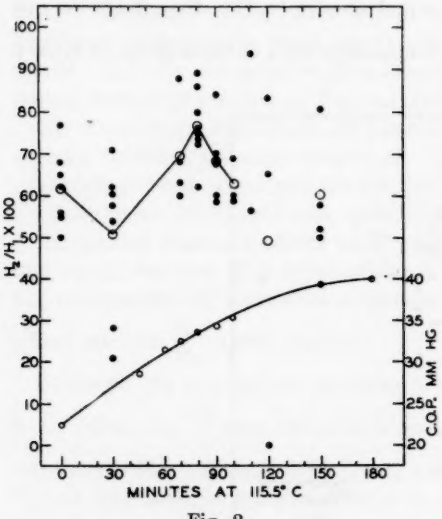

Fig. 2

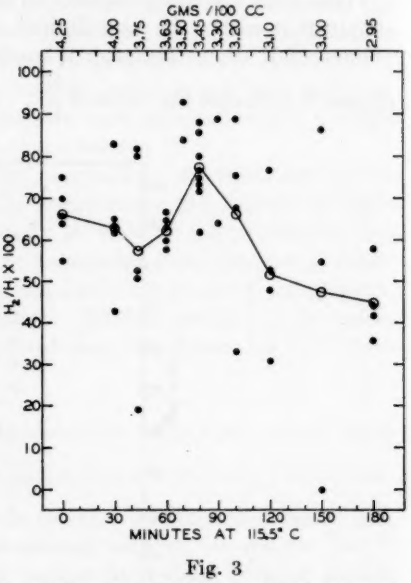

Fig. 3

Fig. 2. The effect of heat treatment (115.5°C.) on the value of 3.45 per cent solutions of gelatin B-78-1 in 0.9 per cent NaCl, as shown from the values obtained for $\frac{H_2}{H_1}$ when R is the heat treated solution. Mean values for the animals receiving a solution are given by the large hollow circles, and are connected to facilitate reading the figure. Small hollow circles on the lower curve give colloidal osmotic pressure (at right) in millimeters of mercury.

Fig. 3. Same as figure 2 except that the concentration of all solutions was adjusted to give colloidal osmotic pressure = 33.5 mm. Hg. The gelatin concentration of the solutions, in grams/100 cc., is given at the top of the figure. The group of animals receiving the 80-minute solution is the same as in figure 2.

of gelatin, as measured by its effect on bleeding volume, when the high molecular weight gelatin is subjected to degradation.

The possibility that some of these changes in value are due to the fact that colloidal osmotic pressure increases progressively in the series (see fig. 2) was investigated by repeating the study with solutions whose gelatin concentration had been adjusted to iso-osmolarity; 33.5 mm. Hg was chosen as the level of colloidal osmotic pressure to be used for this purpose, since the scatter in the data appeared to be least at this level (fig. 2). Data on the iso-osmolar series are summarized in figure 3, which also shows the concentration, in grams/100 cc.,

of each solution. None of the characteristics of the first series appears to have been significantly altered by adjustment of concentration to this level of colloidal osmotic pressure.

Discussion. The data given here supplement those given in the previous report by showing that the change in bleeding volume under these experimental conditions is a function of the nature of the replacement fluid R. The second bleeding volume is evidently not simply a measure of the volume of water which has been given to the animal after the first measurement. The significant differences in $\frac{H_2}{H_1}$ when R is a colloidal solution and when it is crystalloidal (NaCl) warrant the tentative conclusion that colloid is a limiting factor for bleeding volume in animals bled to these levels and given replacement fluids.

Comparison of the data obtained with cell-free and with cellular fluids prepared from blood suggests that the blood cells are not a limiting factor in animals who have lost blood in these volumes (20 cc./kgm. on preparatory bleeding, plus the volume of H_{1a}). Studies which are now in progress suggest that they may become a factor if the end-point for the first measurement is lowered.

That the type of colloid used is important for the second bleeding volume is shown by comparing the data on the hematogenous fluids with the data on gelatin, and by examination of the values obtained with different grades of gelatin in iso-osmolar solution. The low values obtained with the more fully degraded gelatins may be due to their diffusibility. The reason for the other changes in value in the degraded series is obscure.

These studies fail to reveal any difference in the effectiveness of plasma and serum such as has been reported when they are used to maintain life for 24 hours in animals subjected to single rapid hemorrhage (Ivy et al., loc. cit.). On the basis of the information available, it is not possible to decide whether real differences exist which do not reveal themselves within the 4-hour period of our studies, or whether the reported differences were due to methods of preparation of the fluids.

SUMMARY

The change in bleeding volume under conditions which have been described previously has been used for comparing certain fluids as blood substitutes. Comparisons were made on the basis of the $\frac{H_2}{H_1}$ ratios obtained when these fluids were used to replace the blood drawn on the first bleeding volume determination. The mean percentage ratios obtained in groups of 7–10 animals, with their standard errors, were: No fluid, 19.6 ± 6.72; autotransfused blood, 73.6 ± 5.23; pooled heparinized blood, 70.7 ± 4.30; pooled heparinized plasma, 68.1 ± 3.12; pooled defibrinated blood, 62.8 ± 5.18; pooled serum, 70.5 ± 5.75; 0.9 per cent NaCl, 37.6 ± 5.69; 4.85 per cent pork skin gelatin in 0.9 per cent NaCl, 60.5 ± 5.67; 3.45 per cent beef-bone gelatin in 0.9 per cent NaCl, 61.1 ± 2.96.

The value of the bone gelatin undergoes complex changes with heat-degrada-

tion at a temperature of 115.5°C., which are observed both when fixed concentrations are used and when the solutions are adjusted to iso-osmolarity. Peak values were obtained with the partially degraded materials.

It is concluded that bleeding volume under these experimental conditions is a function of the amount and type of colloid in the replacement fluid, and that the cellular content of the replacement fluid is not a limiting factor.

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THE EFFECT OF THE SODIUM CHLORIDE INTAKE ON THE WORK PERFORMANCE OF MAN DURING EXPOSURE TO DRY HEAT AND EXPERIMENTAL HEAT EXHAUSTION[1]

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In contrast to the extensive work on the rôle of sodium chloride in the pathogenesis and treatment of heat cramps (1), the relationship of the NaCl intake to heat exhaustion and cardiovascular function of men working in heat has received only incidental attention (2, 3). This communication will deal with the effects of work in the heat on cardiovascular and related functions and the incidence of heat exhaustion. Thirty-four normal young men lived and worked at high temperatures under rigidly controlled conditions on three different levels of NaCl intake. Reference will also be made to 15 additional subjects who were studied during 92 subject days. The variables measured were pulse rates in rest and work, rectal temperature, rate of sweating, cardiovascular postural adjustment tests, the concentration of chloride in sweat, plasma and urine, blood urea nitrogen and in some cases the refractive index increment of the plasma.

METHODS AND MATERIALS. Details of the regimen under which the subjects ate, slept and worked in a controlled temperature suite have been given in a previous report (4). In brief, control observations were obtained when the dry bulb temperature was 80°F. and the wet bulb 65°F. Hot conditions were represented by a dry bulb temperature of 120°F. and wet bulb 85°F. Night conditions, 6 p.m. to 8 a.m., during the hot period were 85° to 95°F. dry bulb, 65° to 75°F. wet bulb. Approximately an hour and a half was required for a complete change in temperature. In all experiments, work consisted of walking on a motor driven treadmill at 3.25 miles per hour at 7.5 per cent grade for 6 ten-minute periods alternating with 10 minute rest periods for each half day. "Work" pulse rates were obtained by counting the pulse for the first 15 seconds after each work period. Rectal temperatures were taken each half day at the end of the third and sixth work periods. Rate of sweating was measured over the third period in the morning and the sixth period in the afternoon. Subjects were weighed nude and dry to ±7 grams. Modified Crampton (4, 5) blood ptosis tests were carried out before breakfast and 20 minutes after the end of work in the afternoon. In all experiments records were kept of water and fluids

[1] The work described in this paper was done in part under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Minnesota. Important financial assistance was also provided by the Nutrition Foundation, Inc., by Swift and Co., Chicago, by the National Sugar Refiners' Association, N. Y., by the National Confectioners' Association, Chicago, and by the Corn Products Research Foundation, N. Y.

consumed and 24-hour urine samples were collected each day. Body weights, with an empty bladder, were obtained and venous blood was drawn each morning before breakfast. Electrocardiograms were taken on subjects who were on the low NaCl intake during the control period, at the end of the experiment and on all subjects who had marked heat exhaustion. Arm-plus-hand sweat was collected in shoulder-length "neoprene" gauntlets during the second and third work periods each morning and afternoon.

Three series of experiments were carried out. The subjects in series I were 22 soldiers of the 710th M.P. Battalion. Ten of these men were studied on a low NaCl intake (5.8 ± 2 grams) and 12 were studied on a moderate (14.7 ± 2 grams) intake. The duration of the hot period was 3½ days preceded by a control period of 24 hours. In series II, six hired male students of the University were studied during 2 hot periods of 2 days each 3 weeks apart. In each period half of the men were on the low and half on the moderate NaCl regimen, the order being reversed in the second experiment. In this way each subject served as his own control. Series III was similar except 6 University subjects alternated on the high (30 ± 2 grams) and the moderate NaCl intakes. Each hot period in series II and III was preceded by a 2-day control period.

All subjects received a standard diet which contained, by daily analysis, 5.8 ± 2 grams of NaCl. This diet supplied 3100 calories and was adequate, according to ordinary standards, in protein and vitamins. The moderate and high NaCl intakes were achieved by giving in addition 8 and 24 grams of NaCl respectively with the meals. Water was allowed ad lib. except during actual observations and work on the treadmill.

Chlorides were estimated by the mercurimetric titration of Cavett and Holdridge (6) and the method of Keys (7). Food and urine were ashed before analysis. Sweat was analyzed directly. The concentration of plasma chlorides was determined on plasma separated from oxygenated blood. Urea analyses were carried out by a modified Folin-Svedburg method. Total solids (8) of the plasma were estimated with a Pulfrich dipping refractometer in a constant temperature water bath at 30°C.

The statistical significance of differences between means of physiological data was evaluated by means of the t-value (9).

Results in work. The average work pulse rates and rectal temperatures for series I are given in table 1. Statistically significant differences between the two groups for work pulse rates demonstrated a definite advantage for the subjects on the moderate NaCl intake. The rectal temperature differences, although not statistically significant, favor the moderate NaCl group. Results of series II are given in table 2A. The average results show the same advantage for subjects on the moderate NaCl intake.

The results of series III are given in table 2B. No significant differences were found between the work pulse or rectal temperature of six subjects consuming 15 grams of NaCl and the same men consuming 30 grams of NaCl a day.

Cardiovascular results in rest. The average values for pulse rates and blood pressures before and after elevation on the tilt table morning and evening are

given in table 3 along with the average Crampton scores. This table does not include those men who failed to complete the day's work or who fainted before observations could be completed. Differences in the pulse rates of the two groups (subject tilted) appeared on the afternoon of the first day in the heat. This difference became significant on the second afternoon and was still apparent on the third afternoon. The only significant difference in the blood pressures appeared on the afternoon of the second day in the heat (subject tilted).

The moderate NaCl group showed an advantage over the low NaCl group by making a statistically significant improvement in Crampton score between the afternoon of the first day and the afternoon of the second day of the hot period; while, in contrast, the low NaCl group failed to show any improvement. The

TABLE 1

The average work pulse rates and rectal temperatures for 10 men on the low sodium chloride (6 ± 2 grams) intake and for 12 men on the moderate sodium chloride (15 ± 2 grams) with the t values of the differences between groups

The 1 per cent level of significance is 2.84

	CONTROL	DAYS OF HOT PERIOD						
		1		2		3		4
		a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.
Work pulse rates								
Low NaCl....	131	158	174	159	165	157	158	154
Moderate NaCl	133	148	157	144	148	139	143	130
Δ.....	2	−10	−17	−15	−17	−18	−15	−24
t value		1.69	2.99	3.04	4.00	3.64	3.80	4.55
Rectal temperature								
Low NaCl....	100.6	100.5	101.2	101.1	101.5	101.3	101.6	100.9
Moderate NaCl.....	100.3	100.7	101.2	100.7	101.1	100.7	101.1	100.3
Δ.....	−0.3	+0.2	0	−0.4	−0.4	−0.6	−0.5	−0.6
t value.....					0.398	0.659	0.413	

significance of differences between the means is given in terms of the t-value in table 4.

Water balance. The water consumption, body weights, urine volumes, total 24-hour sweat volumes and rate of sweating during work are presented in table 5 for the two groups in series I. The men on the low NaCl intake lost more than twice as much weight, drank less water and sweated less than those on the moderate chloride intake. The two groups produced sweat at approximately the same rate on the first day. However, the low NaCl group failed to drink an adequate amount of water and ended the first 24 hours with a dehydration of 2.5 per cent of the body weight as compared to only 0.77 per cent body weight loss for the moderate chloride group. This was associated with a marked net

loss of NaCl (−8.39 grams) in the low NaCl group (see below) and only a slight net loss (0.53 grams) for the moderate group. The failure of the thirst mechanism in the low NaCl group to demand adequate water is in agreement with other observations (10, 11).

On the second and third days the rate of sweating and the 24-hour sweat production fell off markedly in the low NaCl group and showed no significant change

TABLE 2A

Average pulse rates and rectal temperatures for 6 men in series II studied in two periods of two days each in the heat while on low and moderate NaCl intakes

	CONTROL	DAYS OF HOT PERIOD			
		1		2	
		a.m.	p.m.	a.m.	p.m.
Work pulse rates					
Low NaCl (6 ± 2 grams)	114	140	151	143	154
Moderate NaCl (15 ± 2 grams)	119	140	148	132	141
Δ	+5	0	−3	−11	−13
Rectal temperatures					
Low NaCl (6 ± 2 grams)	100.1	100.3	100.7	100.8	101.2
Moderate NaCl (15 ± 2 grams)	100.0	100.2	100.6	100.1	100.6
Δ	−0.1	−0.1	−0.1	−0.7	−0.6

TABLE 2B

Average pulse rates and rectal temperatures for 6 men in series III studied in two periods of two days each in the heat while on moderate and high NaCl intakes

	CONTROL	DAYS OF HOT PERIOD			
		1		2	
		a.m.	p.m.	a.m.	p.m.
Work pulse rate					
Moderate NaCl (15 ± 2 grams)	107	136	148	134	143
High NaCl (30 ± 2 grams)	103	136	147	132	139
Δ	−4	0	−1	−2	−4
Rectal temperature					
Moderate NaCl (15 ± 2 grams)	100.1	100.6	101.2	100.5	100.9
High NaCl (30 ± 2 grams)	99.8	100.3	101.2	100.3	100.7
Δ	−0.3	−0.3	0.0	−0.2	−0.2

in the moderate NaCl group. The reduction in the total sweat volume of the low NaCl group is accounted for by reduced sweat loss during work.

NaCl metabolism. Table 6 gives the estimated NaCl balances of the two groups in series I. The following assumptions were made in preparing this table: 1, the difference between the NaCl intake and NaCl loss in the urine appeared in the sweat; 2, the changes in body weight represented a loss or gain of a

TABLE 3

The average systolic blood pressures and pulse rates before and after elevation on a tilt table and the average modified Crampton scores of 10 men on the low (6 ± 2 grams) NaCl regimen compared with 12 men on the moderate (15 ± 2 grams) NaCl regimen before and during a 8½ day exposure to dry heat

A.M. values were obtained before breakfast and p.m. values 20 minutes after the last work period in the afternoon

DAY	TIME	DIET	NO. OF MEN	BLOOD PRESSURE		PULSE RATE		CRAMPTON SCORE
				Before	After	Before	After	
Control	p.m.	Low	10	124	115	73	96	56
		Moderate	12	121	112	77	96	60
				—	—	—	—	—
				+3	+3	−4	0	−4
	a.m.	Low	10	120	118	63	87	63
		Moderate	12	122	117	77	83	66
				—	—	—	—	—
				−2	+1	−14	+4	−3
I (hot)	p.m.	Low	7	117	99	98	128	41
		Moderate	12	113	100	94	118	46
				—	—	—	—	—
				+4	−1	+4	+10	−5
	a.m.	Low	10	119	109	68	93	53
		Moderate	12	121	111	73	91	61
				—	—	—	—	—
				−2	−2	+5	+2	−8
II	p.m.	Low	9	115	102	96	131	44
		Moderate	11	120	113	90	108	61
				—	—	—	—	—
				−5	−11	+6	+23	−17
	a.m.	Low	10	123	112	66	96	45
		Moderate	11	118	109	70	90	62
				—	—	—	—	—
				+5	+3	−4	+6	−17
III	p.m.	Low	6	120	110	89	121	46
		Moderate	11	119	109	82	113	56
				—	—	—	—	—
				+1	+1	+7	+8	−10
IV	a.m.	Low	10	121	109	66	88	54
		Moderate	11	114	113	69	91	66
				—	—	—	—	—
				+7	−4	−3	−3	−12

NaCl solution of equal weight whose NaCl concentration was equivalent to the NaCl concentration measured in the plasma. This second assumption that all

TABLE 4

Analysis of the results of the postural adjustment tests in terms of the t-value

VARIABLE	TIMES OR CONDITIONS COMPARED	DEGREES OF FREEDOM	t VALUE CALCULATED	t VALUE AT 5 PER CENT LEVEL OF SIGNIFICANCE
Blood pressure subject tilted	2nd p.m. hot period "Low" vs. "Mod." chloride	18	2.27	2.10
Pulse rate subject tilted	2nd p.m. hot period "Low" vs. "Mod." chloride	18	5.34	2.10
Crampton score	"Low" chloride group 1st p.m. vs. 2nd p.m. of hot period	8	0.33	2.31
Crampton score	"Mod." chloride group 1st p.m. vs. 2nd p.m. of hot period	10	3.77	2.26

TABLE 5

Average morning body weights, water consumption, urine volume, total 24 hour sweat volume and rate of sweating during work of the low chloride group and the moderate chloride group (series I)

VARIABLE	NaCl INTAKE	CONTROL	DAYS OF EXPOSURE TO HEAT		
			1	2	3
Average body weight*	"Low"	157.4	153.4	152.2	151.8
Per cent Δ from control			2.5	3.3	3.6
Average body weight*	"Mod."	154.0	152.8	151.7	152.6
Per cent Δ from control			0.77	1.5	1.3
Water intake (liters)	"Low"		4.82	5.51	5.61
	"Mod."		5.73	6.27	6.33
Urine volume (liters)	"Low"		0.486	0.687	0.670
	"Mod."		0.656	0.648	0.491
Total sweat volume (liters)†	"Low"		6.44	6.12	5.87
	"Mod."		6.37	6.87	6.27
Rate of sweating in work (gms. per minute)	"Low"	a.m.	14.8	12.0	11.1
		p.m.	11.6	10.7	10.4
	"Mod."	a.m.	14.4	14.7	15.8
		p.m.	14.7	15.0	15.0

* Values for body weight represent values with an empty bladder before breakfast after completion of specified number of days' exposure to heat. Body weights expressed in pounds.

† Sweat loss equals water drunk plus water in food plus water metabolism minus the sum of urine water plus fecal water plus water lost through the lungs, corrected for 24 hour weight change.

weight losses are from the extra-cellular fluid phase results in maximal figures for the estimated 24-hour NaCl loss in sweat and from all sources.

The estimated 3-day NaCl deficit for the low NaCl group was 12.71 grams as compared to only 2.84 grams for the moderate NaCl group. The evidence gained from series III appears to indicate that NaCl deficits of 2 to 3 grams have no appreciable effect on the ability of men to perform work in the heat. The low chloride group showed a marked conservation of NaCl when compared to the moderate group, losing only 29.65 grams of salt in three days as compared to 44.78 grams for the men on the higher NaCl intake.

Results in blood and plasma. Table 7 gives the findings of the measured blood constituents in the two groups in series I. The total plasma solids as measured by the refractive index increased 15.2 per cent on the morning of the second day

TABLE 6

The estimated NaCl balances of the low and moderate NaCl groups in series I (see text)

All figures are average grams of NaCl per 24 hours, except weight change which is in kilograms. The numbers in the vertical column heads refer to days of hot period.

	LOW NaCl GROUP			MODERATE NaCl GROUP		
	1	2	3	1	2	3
NaCl intake	5.30	5.84	5.80	15.45	13.19	13.20
Urine NaCl	3.60	1.20	0.45	5.20	2.95	2.45
Weight change (kgm.)	−1.36	−0.54	−0.18	−0.54	−0.54	+0.14
Equivalent NaCl loss*	8.39	3.36	1.04	3.63	3.15	+0.84
Sweat NaCl loss†	10.09	8.27	6.04	13.88	13.39	9.91
Total NaCl loss‡	13.69	9.47	6.49	16.08	16.34	12.36
NaCl balance	−8.39	−3.63	−0.69	−0.53	−3.15	+0.84

* Calculated on the assumption that a gain or loss of weight is due to a gain or loss of extra-cellular fluid. The concentration of chloride in the extra-cellular fluid phase was calculated from plasma chloride concentration.

† Sweat NaCl loss = NaCl intake − urine output ± the NaCl gain or loss equivalent to the weight change.

‡ Total NaCl loss = sweat NaCl loss + urine NaCl loss.

in the low NaCl group but there was later a tendency to return to normal in spite of progressively increasing dehydration. The slight decrease in the hemoglobin suggests blood destruction in the low NaCl group. The increase in blood urea nitrogen in the low NaCl group was accompanied by a 12 per cent increase in daily urinary urea excretion; in contrast the moderate NaCl group showed a 35 per cent reduction in urea eliminated in the urine. The significance of these findings is not clear.

Sweat chlorides. Lehmann (12) has reported a linear relationship between rectal temperature and the concentration of chloride in the sweat. The present experiments were not strictly comparable but they offer small support to this thesis. The average results on 12 men on the moderate NaCl intake (series I) show at best only a rough correlation: $R = 0.655$. The low NaCl group (series I) showed an inverse relationship: $R = -0.790$.

In the experiments reported here there was a marked decrease in the concentration of chloride in the sweat in all groups during the first days of exposure. The average concentration of chloride in the sweat on the morning of the fourth day in the heat was 55 per cent and 38 per cent of the average concentration of chloride in the sweat on the morning of the first day in the moderate and low NaCl groups (of series I) respectively.

Heat exhaustion. Seven of the 49 men who took part in these experiments suffered heat exhaustion of various grades. The symptoms and objective findings are presented in table 8.

Three other cases are included from groups under substantially the same conditions but not reported here in detail. In all there were 39 men on the moderate NaCl regimen and of these only one suffered definite heat exhaustion and

TABLE 7

The concentration of hemoglobin and urea nitrogen in the blood, chlorides in plasma and the refractive index increment of plasma of the low and moderate chloride groups in series I

DETERMINATION	DIET GROUP	CONTROL	DAYS* OF EXPOSURE TO HEAT		
			1	2	3
Refractive index increment†	Low chloride	0.0171	0.0197	0.0187	0.0183
	Moderate chloride	0.0173			0.0175
Hemoglobin (grams per 100 cc.)	Low chloride	16.02	15.7	15.8	15.7
	Moderate chloride	15.4			15.7
Plasma chlorides (mgm. NaCl 100 cc.)	Low chloride	617	613.2	585	582
	Moderate chloride	637			635
Blood urea nitrogen (mgm. per 100 cc.)	Low chloride	12.0	19.8	20.6	20.7
	Moderate chloride	13.8			14.7

* Values represent findings in blood drawn in morning before breakfast after completion of specified number of days' exposure to heat.

† Refractive index of plasma minus the refractive index of water.

prostration while one other man had to interrupt the work schedule because of mild heat exhaustion. In contrast, 5 men of the 20 on the low NaCl regimen suffered heat prostration and 3 others suffered heat exhaustion requiring interruption of the work schedule. The one man (Du) who developed heat prostration in the moderate NaCl group was 46 years old and the oldest man studied. Du had a very high rate of sweat production. Du lost 23.4 grams of sweat per minute in work as compared to an average of 14.9 grams/min. for all men in the moderate NaCl group. He showed a net loss of 5.5 per cent of his body weight on the first day in the heat and as a result he was badly dehydrated when he collapsed during the morning of the second day.

Case Wa is interesting because he represents heat exhaustion which occurred during a simultaneous increase in the rate of sweating. The routine rate of

TABLE 8

The symptoms, pulse rate, rectal temperature, blood pressure, blood hemoglobin and plasma chlorides of 10 men who suffered various grades of heat exhaustion during a 3½ day exposure to heat

All subjects except Du were on the low chloride regimen

SUBJECT	DAY OF HOT PERIOD	SYMPTOMS	WORK PULSE RATE	WORK RECTAL TEMP.	RESTING PULSE RATE	RESTING PRESSURE		BLOOD HEMOGLOBIN	PLASMA CHLORIDES
								grams per cent	*mgm. per cent*
Dar	p.m. 1st	Nausea, vomiting, vertigo, headache	204	102	120	116/70			584
Cel	p.m. 2nd	Nausea, vomiting, vertigo, very slight cramps	156	101			a.m. ill	16.9 18.2	593 567
He	p.m. 1st	Refused to continue, complained of dyspnea	180	102.8					
Ma	p.m. 3rd	Nausea, vomiting, vertigo, weakness	168	100.2	88	96/70	a.m. ill	15.9 17.0	570 618
Wa	a.m. 1st	Fainted before going on treadmill. Fourth period rate of sweating 32/grams/min.*	140	99.2			a.m. ill	17.0 16.55	629 620
Tw	p.m. 2nd	Refused to continue, slightly hysterical	172	101.6			a.m. ill	14.5 14.5	615 609
Gol†	a.m. 3rd	Vomiting, nausea			104	108/90			
Be†	p.m. 3rd	Vomiting, nausea vertigo, jaundice							
Du‡	a.m. 2nd	Vomiting, vertigo, nausea, marked weakness	160	101.6	116		a.m. ill	15.6 20.07	640 610
Ne§	p.m. 1st	Weak, refused to continue	174	103.6	132	122/60			

* Five subsequent determinations of rate of sweating averaged 12.8 gms./min.

† These men took part in preliminary low chloride experiments and are not included in series I, II, or III.

‡ Forty-six years old; on the moderate chloride diet.

§ Took part in an 8 day experiment previously reported in detail Taylor, Henschel and Keys, Report to N.R.C. April, 1943. This Journal, 1943. (In press.)

sweating was measured over the third period of work on the morning of the first day in heat and was found to be 32 grams/min. At the beginning of the fourth period he fainted as he was walking up the 3 steps to the treadmill platform. Five subsequent determinations of the rate of sweating on Wa when he was able to resume work averaged 12.8 grams/min. It should be noted that Wa had a milder though similar episode in the afternoon of the same day with a normal rate of sweating.

Electrocardiograms were obtained on all men during the control period and at the end of the experiment. Records were also obtained on all men suffering from heat exhaustion. In no case did the low plasma chloride influence the electrocardiographic pattern.

The heat exhaustion encountered in this Laboratory differed from the "dehydration exhaustion," reported by Adolph (13) on subjects working at high temperatures without water, in that nausea and vomiting were a prominent feature, occurring in 60 per cent of our cases while Adolph reported 35 cases of dehydration exhaustion without either nausea or vomiting. McCance (14) has reported that nausea and vomiting occur in uncomplicated salt deficiency.

In a previous report (4) it has been shown that heat exhaustion is associated with the failure of men to acclimatize (as measured by the pulse rate) and with a poor cardiovascular postural adjustment test in the evening preceding the actual development of symptoms. It was also pointed out that food (with salt), water and rest (subjects remaining in the heat) are the only measures that are ordinarily necessary to restore to activity the men who develop heat prostration.

Discussion. The evidence presented here indicates that men working in the heat, sweating 5 to 8 liters a day do not require more than 15 to 17 grams of salt a day. The total 24-hour sweat volumes are similar to those measured in the desert by Dill *et al.* (17), in mines by Moss (2) and recently by ourselves in desert maneuvers. Salt requirements for tropical conditions also may be estimated on the basis of these experiments since Lee *et al.* (15) have shown that for a given effective temperature (Houghten and Yaglogou, 16) the salt loss during work is less in the hot wet conditions than in the hot dry conditions. Thus it may be expected that men sweating 5 to 8 liters in the tropics will not require more than 15 to 17 grams of NaCl a day.

It is apparent that the salt requirement may be best judged by the physiological response of the individual. For this reason, we wish to emphasize the results of series III in which no significant physiological advantage was gained by increasing the NaCl consumption from 15 to 30 grams a day during work in the heat.

The relationship of cardiovascular function to heat exhaustion has been recognized by other investigators (Weiner, 18; Keeton *et al.*, 19; Ferris *et al.*, 20). A number of observers, who were primarily concerned with the study of heat cramps, have noted without giving data that when adequate NaCl is supplied to miners (Moss, 2) or steel workers (Dill *et al.*, 3) subjective fatigue is reduced and the incidence of heat exhaustion lowered. Clear evidence is submitted here to show that a salt deficit imposes a severe strain, in addition to the task of heat regulation, on the cardiovascular system of men performing work in high environ-

mental temperature and that this additional burden will limit the amount of work performed and in some cases lead to the development of heat exhaustion. The cause of this effect appears to be a contracted circulating blood volume. The extent of the reduction of the blood volume in these experiments may be estimated from the NaCl loss. The estimated NaCl loss of the low NaCl group for the first 24 hours was 8.39 grams. If the chloride all came from the extracellular fluid, this would represent 1.74 liters. This is 10.3 per cent of 16.87 liters, the average extracellular fluid found by Forbes, Dill and Hall (21) in 10 men. This change is the same order of magnitude as the per cent change in the refractive index increment of the plasma (15.2 per cent), a figure which may be taken as roughly proportional to the blood volume change.

Heat exhaustion appears to be circulatory failure or impending circulatory failure during activity. The subject cannot perform continuous physical work, indeed he may faint while attempting it, but if he ceases physical activity and lies down the capacity of his cardiovascular system is adequate to meet the requirements of resting metabolism. Circulatory failure can be brought on by placing excessive demands on the heat regulating mechanism (18). The more common situation appears to result from the integrated effects of a contracting blood volume and the demands of heat regulation for circulation through the skin. The blood volume may be reduced by failure to drink enough water to keep up with the water lost by sweating (13) or by failure to consume enough chloride to replace the salt lost in sweat production (present results).

Talbott (1) has maintained that the lowering of sodium and chloride ions in serum is the principal etiological factor in the pathogenesis of heat cramps. Both the concentration of chloride in plasma and the total chloride loss of the subjects on the low NaCl diet were well within the range reported as being characteristic of heat cramps. Talbott and Mickelsen (22) reported 5 cases of heat cramps with an average serum chloride of 96.9 M.E./liter on admission. Eight subjects of the 20 studied on the low NaCl diet had plasma chloride concentrations of less than 100 M.E./liter, the average being 94.6 and the lowest 89 M.E./liter. None of these men had real heat cramps but 5 of them suffered heat exhaustion. One man, C. E., complained of slight twitchings in the right ankle and hip but this could hardly be termed true heat cramps. These observations serve to emphasize that no one factor accounts for the development of true heat cramps (3). Dill *et al.* (3) have suggested that the severity of heat cramps depends both on the intensity of the work and the degree of salt loss. It is probable that the intensity of work in these experiments was not as great as that encountered for brief periods in steel mills and mines although the total work output cannot be much inferior judging from pulse rates. In any case it is clear that NaCl loss *per se* and the resulting reduction of plasma chlorides will not necessarily result in heat cramps but will lead in many cases to heat exhaustion.

SUMMARY

The effects of 3 levels of NaCl intake on cardiovascular functions were studied in 49 "normal" men in work and rest during exposure to hot, dry conditions.

The salt intakes were, in grams NaCl per 24 hours, 6 ± 2 ("low"), 15 ± 2 ("moderate") and 30 ± 2 ("high"). Day temperature was 120°F. dry bulb, wet bulb of 85°F., night 85° to 95°F. dry bulb, 65° to 75° wet bulb. Control studies were at 80°F. dry, 65°F. wet bulb. Diet, exercise and other conditions were rigidly standardized. Water was allowed *ad lib.* Periods in the heat ranged from 2 to 8 days each.

Pulse rates, rectal temperature, sweat composition and rate of sweating were studied in work and in rest. Blood ptosis tests were made. Sweat production ranged from 5 to 8 liters per day.

No advantage in any of the variables measured was demonstrated for men on a "high" daily intake of 30 grams NaCl as compared to the "moderate" (15 grams) intake.

Men maintained on a "low" (6 grams) intake of salt had higher pulse rates and rectal temperatures in work than men on a "moderate" salt intake. The deleterious effect of the "low" salt intake was also reflected in poorer postural cardiovascular adjustment. The men on the "low" salt intake lost more than twice as much body weight, drank less water and sweated less than the men on the "moderate" salt intake.

The "low" salt intake resulted in an average net deficit of 13 grams NaCl for 3 days in the heat. The men on the "moderate" salt intake appeared to be in NaCl balance after 3 days in the heat.

Heat exhaustion and prostration, characterized by nausea, vomiting, tachycardia, hypotension, vertigo, dehydration and collapse, occurred in 25 per cent of the men on the "low" salt intake and in only 2.5 per cent of the men on the "moderate" salt intake. Rest in the heat, food (with salt) and water sufficed to restore all these men so that they could continue work in the heat in 8 to 24 hours.

Although pronounced hypochloremia was observed in many instances heat cramps did not occur.

There was little or no relation between the concentration of chloride in the sweat and the rectal temperature.

It is concluded that heat exhaustion and ability to work in the heat are almost wholly dependent on cardiovascular function and that a moderate salt intake is more important to preserve this function than to prevent heat cramps. Hypochloremia is not the only factor in heat cramps.

It is further concluded that the NaCl requirement of unacclimatized men who are sweating 5 to 8 liters a day is not greater than 13 to 17 grams daily. An increase in salt intake above this level results in increased loss of salt and water in the urine with no apparent advantage.

Acknowledgments. Completion of this work would not have been possible without the careful attention to detail and willing help of Miss Angie Mae Sturgeon, Head Technologist, Miss Evelyn Pearson, Dietitian, Mrs. Nedra Foster, Assistant Dietitian and Mr. Howard Condiff, Assistant Chemist. We also wish to thank Dr. Josef Brozek for assistance in the statistical analysis of the data.

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EFFECTS OF PURIFIED ANTERO-PITUITARY HORMONES ON THE CARBOHYDRATE STORES OF HYPOPHYSECTOMIZED RATS[1]

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It has been recognized for some time that antero-pituitary hormones are needed to enable hypophysectomized rats to withstand fasting periods without marked loss of carbohydrate stores. It has been demonstrated that adrenal cortical steroids (3, 16) or a crude adrenocorticotropic hormone (4) will preserve or increase blood sugar, liver glycogen and muscle glycogen levels of fasted hypophysectomized rats. If a crude anterior lobe extract is given throughout the fasting period, complete maintenance of muscle glycogen levels may be produced without effects on blood sugar or liver glycogen. This effect may also be obtained in the absence of both adrenals (11). The pituitary principle which produces this effect has not been identified with any of the known anterior lobe hormones and has been termed the glycostatic hormone (1, 2).

By employing the most purified anterior hypophyseal hormone preparations now available it was hoped that a long deferred answer might now be given the question "Is myoglycostatic activity a property of one or of several of the six known antero-pituitary hormones?" In the course of these studies concordant observations were frequently made on the hepatic glycogen and blood sugar.

EXPERIMENTAL CONDITIONS, METHODS AND MATERIALS. In most of these experiments, conditions were similar to those proposed by Russell for the assay of the myoglycostatic hormone (2). Male rats, hypophysectomized when 2–3 months old, were used 2–4 weeks postoperative. In part of the experiments, immature females, operated upon at the age of 26–28 days were employed 5–10 days postoperative, but occasionally as long as 3.5 months after the operation. In each experiment only animals of the same age and postoperative period were used, generally in groups of 5–8 rats. All rats were fasted for a 24 hour period, started at 1 p.m. and received the hormone intraperitoneally in three doses at 24, 10–12, and 4 hours before sacrifice. During longer injection periods the hormone was given once daily save on the last day when the schedule mentioned above was followed. Thus in a series of experiments, rats were treated for 3–4 days, starting two weeks postoperative, in another series for 9–21 days, starting on the day following operation.

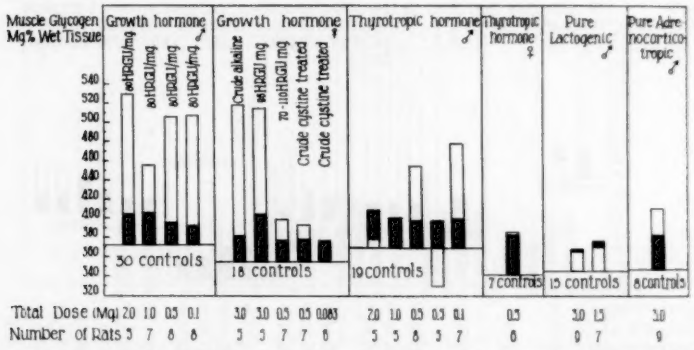
The hormone preparations while in general similar to those used in previous metabolic studies (5, 6, 7), embraced two highly purified substances—the adrenocorticotropic and lactogenic hormones. The thyrotropic preparation contained

[1] Aided by grants from the Research Board of the University of California and the Rockefeller Foundation, New York City.

slight amounts of the interstitial cell stimulating hormone and an uncertain amount of growth hormone.[2]

All rats were anesthetized with sodium amytal for the collection of blood and tissue samples. The liver and muscle glycogens were determined by a minor modification of the Cori method (8) used in this laboratory for several years. This consists in proceeding after the acid hydrolysis without filtration. This was demonstrated by control experiments to be non-essential. The glucose determinations were performed by the method of Benedict (9). When an ignited molybdic anhydride was used in preparing the color reagent, a very stable color was obtained, reaching a color maximum in 30 minutes which changed less than 2 per cent during 8 hours. The Sheard-Sanford photoelectric colorimeter was used. Blood was deproteinized by the copper-tungstate method of Somogyi (10).

DISCUSSION OF RESULTS. The data are presented in graphic form to show the



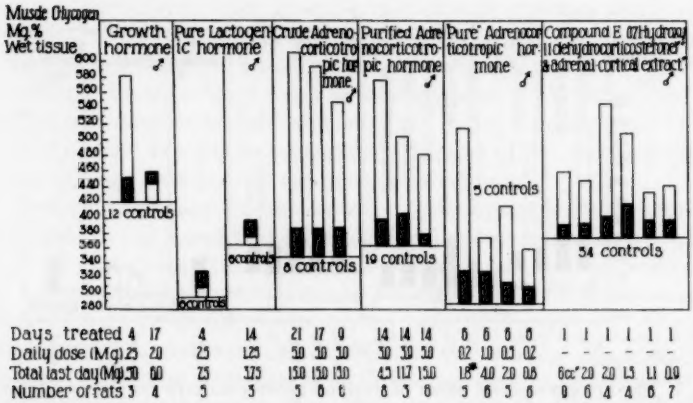
Graph I. The myoglycostatic effect of purified anteropituitary hormones administered during fasting in three divided doses at the 24th, 10–12th, and 4th hours before sacrifice of hypophysectomized rats.

increase of the experimental groups over their respective controls in graphs I and II; the black areas indicate the standard deviation[3] of the difference and an increase of two times this was considered significant. When Russell (1, 2) studied and compared the ability of various pituitary fractions to maintain muscle glycogen in fasted hypophysectomized rats, she found growth promoting extracts always to be potent in this respect. Since then methods have been developed for a better separation of the growth principle from the other known hormones. When such improved preparations were tested for myoglycostatic

[2] We desire to thank heartily Dr. Walter Marx for many samples of a highly purified and potent growth hormone, Drs. William R. Lyons and C. H. Li for completely purified lactogenic hormone, Dr. C. H. Li for completely purified adrenocorticotropic hormone and Jane Fraenkel-Conrat for thyrotropic preparations which had been purified to a fargoing extent. We especially desire to thank Dr. Heinz Fraenkel-Conrat for the suggestion that these studies be undertaken and for his kindness throughout their execution and formulation.

[3] Standard deviation of the difference between the mean of the controls and the mean of the experimental groups.

activity, they were still found to be effective. About ten different preparations of growth hormone were given to a total of 64 rats and all groups treated with the hormone showed increased muscle glycogens when compared with the controls (graph I). One milligram of various preparations caused increases of 50–90 mgm. per cent. In one experiment as little as 0.1 mgm. of a highly purified growth hormone preparation caused statistically significant maintenance, but higher doses of this preparation were not appreciably more effective (graph I). This is not very surprising in view of Russell's finding of a very narrow range in which a clear-cut dose-response relationship could be observed (2). Treatment with the hormone for 3 or 4 days preceding the fast appeared no more effective than the usual one day injection period, while after 17 days of hormone treatment no appreciable effect was noted (graph II). (Confirmation of L. L. Bennett, 11.)


Muscle Glycogen
Mg.%
Wet tissue
Growth hormone
Pure Lactogenic hormone
Crude Adrenocorticotropic hormone
Purified Adrenocorticotropic hormone
Pure Adrenocorticotropic hormone
Compound E (17Hydroxy 11dehydrocorticosterone) & adrenal cortical extract
12 controls
6 controls
6 controls
8 controls
19 controls
5 controls
34 controls
Days treated 4 17 4 14 21 17 9 14 14 14 6 6 6 6 1 1 1 1 1 1
Daily dose (Mg.) 2.5 2.0 2.5 1.25 3.0 3.0 3.0 3.0 3.0 3.0 0.2 1.0 0.5 0.2 – – – – – –
Total last day (Mg.) 3.0 6.0 2.5 3.75 15.0 15.0 15.0 4.5 11.7 15.0 1.6* 4.0 2.0 0.6 6cc* 2.0 2.0 1.5 1.1 0.9
Number of rats 3 4 5 5 5 6 6 8 3 6 5 6 3 6 9 6 4 4 6 7

Graph II. The myoglycostatic effect of purified anteropituitary hormones administered daily, and in three divided doses at the 24th, 10–12th, and 4th hours before sacrifice, on the last day of fasting.

* Injected nine times, at two hour intervals, during the last day of treatment (other ACTH groups received four injections, at 3 hour intervals, during the last day of treatment).

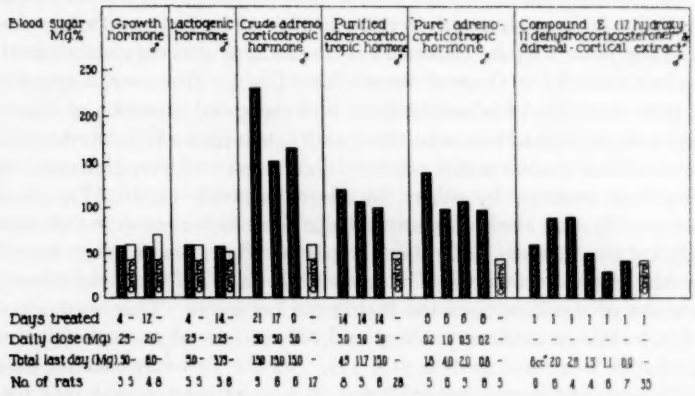
In contradistinction to the effectiveness of growth hormone preparations in maintaining muscle glycogen, they did not maintain blood sugar (graph III) or liver glycogen under the same conditions.

Thyrotropic extracts were generally found to be rich in glycostatic activity in older studies (2). Forty-four hypophysectomized rats were therefore injected with a number of purified thyrotropic preparations. Results indicated that there was no statistically significant maintenance of muscle glycogen in 5 out of 7 experiments. The two groups in which maintenance did occur might be accounted for by the contamination with growth hormone.

Pure adrenocorticotropic hormone (ACTH) (12) was tested at one level in the usual test (graph I). When 3 mgm. were given, muscle glycogens were found 65 mgm. per cent above controls, the difference not being significant.

The previous experiments with less pure adrenocorticotropic, gave similar small increases. In a considerably greater number of experiments, however, ACTH was given for periods ranging from three to twenty-one days, starting the treatment in many experiments on the day following the operation and in others from two weeks to three and a half months postoperative (graph II). In all these experiments muscle glycogens were maintained. Blood sugar determinations in these groups paralleled muscle glycogen values strikingly, the blood sugars ranging up to 233 mgm. per cent in the treated groups (graph III). The highest muscle and blood carbohydrate values were obtained with a crude ACTH. In contradistinction to the finding of Bennett (4), liver glycogen though found to be slightly increased in these experiments was never super-normal.

In view of the general belief that the hormonal control of blood sugar and liver glycogen depends on the adrenal and that these values are generally correlated,


Blood sugar Mg%
Growth hormone
Lactogenic hormone
Crude adreno corticotropic hormone
Purified adrenocortico tropic hormone
Pure adreno-corticotropic hormone
Compound E (17 hydroxy 11 dehydrocorticosterone) adrenal-cortical extract
250
200
150
100
50
0
Control
Days treated 4 - 12 - 4 - 14 - 21 17 0 - 14 14 14 - 6 6 6 6 - 1 1 1 1 1 1 1
Daily dose (Mg) 25- 20- 25- 125- 50 50 50 - 3.0 3.0 3.0 - 0.2 1.0 0.5 0.2 - - - - - - - -
Total last day (Mg) 50- 80- 50- 375- 150 150 150 - 4.5 11.7 15.0 - 1.0 4.0 2.0 0.8 - 8cc 2.0 2.0 1.5 1.1 0.8 -
No. of rats 3 5 4 8 5 5 3 6 5 6 6 17 6 5 6 28 5 6 5 6 5 8 6 4 4 6 7 35

Graph III. The blood sugar levels of hypophysectomized rats given anteropituitary hormones daily and three times during the last day while fasting.

while muscle glycogen is only secondarily influenced by this gland (2, 4, 5, 7) many control experiments were performed to verify the surprising findings here reported; namely, that stimulation of the adrenal had only minor effect on liver glycogen, while causing hyperglycemia and increased muscle glycogen values. When the analytical technique was tested repeatedly by the determination of known amounts of hog liver glycogen added to rat livers, recovery was regularly found to be above 87 and generally close to 92 per cent, if the following precautions were observed: 1, the tubes containing 30 per cent KOH were placed into a boiling water bath immediately after the addition of the freshly extirpated livers; 2, sugar determinations were carried out immediately after acid hydrolysis of the liver glycogen. In some of the earlier experiments, before the importance of these precautions was realized, one secured approximately 0.10 per cent lower liver glycogen values. However, experimental and control rats were treated alike in each case and since recent experiments in which this error was avoided

confirmed the findings of the earlier ones, no significance can be attributed to this factor in the interpretation of the results.

It appeared of importance to re-study the effect of adrenal extracts and crystalline steroids on the glycogen and sugar stores of hypophysectomized rats. Six groups of rats were treated with 17-hydroxy-dehydrocorticosterone (compound E of Kendall[4]) during the 24 hour fast with doses ranging from 0.4 mgm. to 2.0 mgm. (graph II). Muscle glycogen values were found increased by all but the lowest level of the hormone, the effect being significant after the administration of more than 1.1 mgm. Blood sugars were also well maintained by 2 mgm. of compound E, with doubtful effects at lower doses (graph III). Liver glycogen again was found low in all experiments never exceeding 0.2 per cent, while the controls were generally about 0.13 per cent. Similar effects were produced by 6 cc. of Upjohn Cortical Extract. Thus cortical extract and compound E were found to act quite similar to ACT in our rats, causing appreciable increases in muscle but not in liver glycogen; in regard to blood sugar the pituitary hormone caused hyperglycemia while the steroid at the dosages used only maintained blood sugars at levels similar to those of normal fasted rats. However, it appears quite possible that the mode of administration and the speed of action of the steroids may here be an important factor and that with continuous administration in larger amounts these compounds would produce blood sugar and liver glycogen increases similar to those reported by others for adrenal steroids (3, 16, 17). It is especially noteworthy that the low doses used significantly increased muscle glycogen.

Myoglycostatic studies have been reported for growth, thyrotropic and adrenocorticotropic hormones. The remaining chemically characterized pituitary hormone of significance is the lactogenic hormone. This hormone can be prepared in a state of uniform potency and our various physico-chemical studies indicate that it is a pure protein (13, 14). In the two experiments presented (graph II) in which it was administered to hypophysectomized rats for more than 24 hours the adrenals did not show an increase in weight and no histological stimulation was evident. When the hormone was given only during the fasting period at dose levels of 1.5 and 3.0 mgm. no significant increases in muscle glycogen were observed (graph I). When it was given for 4 and 14 days to two other experimental groups at levels of 2.5 and 1.25 mgm. daily with three times this dose during the fasting period there likewise was no significant increase in the muscle glycogen (graph II). Throughout the experiments with lactogenic hormone liver glycogen and blood sugars were uniformly low.

The data presented in graph I show that no greater effects were obtained with a purified cysteine-treated growth hormone preparation than with a crude alkaline extract. Euglobulin and pseudoglobulin fractions of a crude extract were found active at 1 mgm. while the albumin fraction showed no activity at 2 mgm. The fact that purified hormone preparations were generally no more active than crude extracts indicated the possibility that the latter might owe their activity to a synergism of some of their component hormones. Therefore a considerable number of hormone combinations were tried in search for this

[4] We desire to thank Dr. E. C. Kendall heartily for this valuable substance.

synergism. When growth hormone was given together with ACTH and lactogenic hormone for one day, the effect was no greater than that produced by the growth hormone alone. When the same combination was given for 3 days, it caused the highest muscle glycogen values (230 mgm. per cent above controls), but the effect was less than additive. Also upon combination of growth hormone with thyrotropic or with 17-hydroxy-dehydrocorticosterone (compound E) no synergism was noted.

Conclusions. Russell found myoglycostatic activity to varying extent in all anterior pituitary extracts tested with the exception of the albumins (growth discard fraction) and a lactogenic preparation (2). Using the same technique, i.e., the 24 hour fasting period with equal amounts of hormone at 24th, 10th to 12th and 4 hours before sacrifice, we find purified growth hormone alone to show pronounced effects at low doses. Russell hesitated to identify the myoglycostatic hormone with the growth hormone, because of the glycostatic activity of other hormone fractions, low in growth activity, and because of the fact that the purification of the growth hormone was not paralleled by an increase in myoglycostatic potency, when compared to crude extracts. The first instance may have been confusing because of adrenotropic hormone contamination inhibiting the growth activity of the extracts (15), and thereby producing extracts apparently low in growth potency. Secondly, we have confirmed the failure of growth preparations to show marked increases in myoglycostatic potency during purification. However, the fact that growth hormone has since been nearly freed from several known hormones (ICSH, FSH, lactogenic, adrenocorticotropic, and thyrotropic) without losing myoglycostatic activity at low dose levels (0.1 to 1 mgm.) indicates that one or both of the two components present by electrophoretic analysis of purified growth fractions might be the myoglycostatic agent, or agents.

The time relationships of the muscle glycogen increases presented in this paper are of the greatest importance.

a. Growth hormone is most effective when given for 1–3 days. It becomes ineffective in maintaining muscle glycogen when given for 2–3 weeks (4).

b. Adrenocorticotropic hormone is only slightly effective or inactive when 3 injections are given during the 24 hour fast; but it is very effective when given daily for one to two weeks. This suggests that dissimilar mechanisms are responsible for muscle glycogen storage by growth and adrenocorticotropic hormones. Maintenance of adrenal cortical size and secretion by adrenocorticotropic hormone gives increased muscle glycogen levels in the hypophysectomized rat (graph II) which are similar to those obtained with 17 hydroxy-11-dehydrocorticosterone (Kendall's Compound E). Similar increases have been previously reported for cortical extract and corticosterone (Kendall's Compound B) by Russell and Craig (3) and Long and Katzin (16). Russell and Craig (3) obtained supernormal muscle glycogen levels when adrenal steroid (Compound B) and "glycostatic" extract were administered simultaneously to hypophysectomized rats.

Two factors are probably instrumental in the failure to obtain supernormal

liver glycogen in fasted hypophysectomized rats given compound E, or cortical extract reported in graph II.

a. The hormone or extract was not given in small hourly doses. Reineke and Kendall (17) have recently emphasized the importance of hourly administration of potent extracts to obtain liver glycogen storage in fasting adrenalectomized rats.

b. The amount of cortical extract just sufficient for the maintenance of normal carbohydrate levels in adrenalectomized rats is without effect in the absence of the hypophysis (Bennett, 18; Grollman, 19). Russell (20) presents an excellent analysis of the interplay of glyconeogenetic action of adrenal cortex hormones and crude anteropituitary extract effects upon glucose utilization rate in the eviscerated rat. The hypophysectomized rat oxidized sugar at a much greater rate than the normal or adrenalectomized rat.

In recent experiments (21) pure adrenocorticotropic hormone administered to hypophysectomized rats fasting during low pressure treatment has not increased their liver glycogen to levels found in anoxic rats with intact hypophyses. In these animals, the adrenals were larger than normal, and blood sugar levels, and muscle glycogen markedly increased by the adrenocorticotropic administration. Bennett (4) using an impure adrenocorticotropic hormone at high dose levels obtained supernormal liver glycogen levels in fasted hypophysectomized rats. Experiments are planned to determine what anteropituitary hormone or hormones are necessary for administration with pure adrenocorticotropic hormone to give supernormal liver glycogens in fasting hypophysectomized rats. Those anteropituitary fractions high in myoglycostatic and growth hormone action are highly effective in depressing the respiratory quotient of glucose fed rats (22, 23, 24). This action if it reduces glucose oxidation in the periphery might conceivably act with adrenotropic hormone to increase the liver glycogen during fasting to supernormal levels.

SUMMARY

1. Myoglycostatic activity is greatest in crude extracts of the anterior hypophysis and in highly purified growth promoting extracts.

2. Thyrotropic extracts known to be impure had activities no greater than to be expected from possible growth hormone contamination.

3. Adrenocorticotropic hormone showed slight or no activity during the one day test in which growth hormone preparations were highly effective. However, when through longer administration of adrenocorticotropic hormone, the adrenal gland becomes normal or hypertrophied, muscle glycogen and hepatic glycogen are maintained by it during fasting.

4. Pure lactogenic hormone is ineffective in the one day myoglycostatic test or when administered for 4 or 14 days.

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